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DATE OF FILING October 16, 2006

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
An, *et al.*

Serial No.: 09/974,546

Filed: October 10, 2001

For: BIOMARKERS AND TARGETS FOR DIAGNOSIS, PROGNOSIS AND MANAGEMENT OF PROSTATE, BREAST AND BLADDER CANCER

Group Art Unit: 1643

Examiner: Rawlings, Stephen L.

Atty. Dkt. No.: UROC:018USD2

APPEAL BRIEF

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APPEAL BRIEF

MS Appeal Briefs
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

Appellants hereby submit this Appeal Brief to the Board of Patent Appeals and Interferences in response to the final Office Action ("the Action") dated November 10, 2005. A Request for an Extension of Time for three months is also enclosed. With this extension, the deadline for submission of this Brief is Monday, October 16, 2006, by virtue of the date (May 15, 2006) stamped on the return postcard filed with the Notice of Appeal on May 10, 2006, and the fact that October 15, 2006, falls on a Sunday. A Request for Oral Hearing is being filed concurrently with this Appeal Brief.

A check for fees in the amount of $1,520.00 for the Appeal Brief ($500.00) and the Request for a Three-Month Extension of time ($1,020.00) is enclosed. Should any additional fee(s) be required, consider this paragraph such a request and authorization to withdraw the

appropriate fee under 37 C.F.R. §§ 1.16 to 1.21 from Fulbright & Jaworski L.L.P. Account No.: 50-1212/UROC:018USD2.

I. REAL PARTY IN INTEREST

The real parties in interest are Urocor, Inc. of Oklahoma City, Oklahoma, the assignee, and LabCorp of America, headquartered in Burlington, North Carolina, which purchased Dianon Systems, Inc., for which Urocor was the predecessor.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

III. STATUS OF THE CLAIMS

Claims 78-94 were originally filed in a Preliminary Amendment on October 10, 2001, while claims 1-77 of the parent application, Serial No. 09/097,199 (now U.S. Patent No. 6,218,529), were canceled.

In a Response to Restriction Requirement dated April 20, 2004, Appellants elected with traverse to prosecute the invention of Group XIX, claims 78-94, drawn to a method for treating cancer comprising administering an agent that inhibits a peptide or polypeptide encoded by SEQ ID NO: 83 or a fragment thereof. In a Response to Notice of Non-Compliant Amendment dated August 16, 2004, Appellants elected the species of invention wherein said cancer is prostate cancer. In an Office Action dated November 22, 2004, Groups XIX and XX (that is, a method for treating cancer comprising administering an agent that inhibits a peptide or polypeptide encoded by SEQ ID NO: 85 or a fragment thereof) were rejoined. Further, in this same Action, the requirement to elect a species of the invention from bladder, breast and prostate cancer was withdrawn.

In a Response to Office Action dated April 21, 2005, claims 78, 83, 86, 87 and 92 were amended and claim 85 was canceled.

Claims 78-84 and 86-94 were pending and rejected in the final office action dated November 10, 2005. Thus, claims 78-84 and 86-94 are the subject of this appeal.

IV. STATUS OF AMENDMENTS

No amendments have been filed since the final office action.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention concerns methods for a method of treating a patient with breast cancer, bladder cancer or prostate cancer comprising administering to the patient an effective amount of an agent that binds to a peptide or polypeptide encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:83 or SEQ ID NO:85, or a fragment thereof. Specification at least at page 11, lines 6-17; page 13, lines 1-3; page 21, lines 24-26; page 44, lines 20-27; page 52, lines 12-23; page 54, lines 13-18 and page 117, lines 4-12.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

- Claims 78-82, 86-91 and 94 have been rejected as lacking adequate written description under 35 U.S.C. § 112, first paragraph.

- Claims 78-84 and 86-94 have been rejected as lacking enablement under 35 U.S.C. § 112, first paragraph.

- Claims 78-82, 86-91 and 94 are rejected as being indefinite under 35 U.S.C. § 112, second paragraph.

VII. ARGUMENT

A. Substantial Evidence Required To Uphold Examiner's Position

Findings of fact and conclusions of law by the U.S. Patent and Trademark Office must be made in accordance with the Administrative Procedure Act, 5 U.S.C. § 706(A), (E), 1994. *Dickinson v. Zurko*, 527 U.S. 150, 158 (1999). Moreover, the Federal Circuit has held that findings of fact by the Board of Patent Appeals and Interferences must be supported by "substantial evidence" within the record. *In re Gartside*, 203 F.3d 1305, 1315 (Fed. Cir. 2000). In *In re Gartside*, the Federal Circuit stated that "the 'substantial evidence' standard asks whether a reasonable fact finder could have arrived at the agency's decision." *Id.* at 1312.

Accordingly, it necessarily follows that an Examiner's position on Appeal must be supported by "substantial evidence" within the record in order to be upheld by the Board of Patent Appeals and Interferences.

B. Claims 78-82, 86-91 and 94 Are Adequately Described

Claims 78-82, 86-91 and 94 are rejected under the first paragraph of 35 U.S.C. § 112 as lacking adequate written description. The Action contends that the claims contain subject matter that was not described in the specification in such a away as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. More specifically, the Action states that the genus of "agents" recited in the claims includes structurally and functionally disparate molecules including, for example, naked

antibodies, that specifically bind to the polypeptide and inhibit its activity or function, such that treatment of cancer cells with the antibody provides therapeutic benefit. The Action then contends that there is no language in the specification that adequately describes the genus of antibodies that bind a polypeptide of the present invention and inhibit its activity or function, so as to provide therapeutic benefit. To support this contention, the Action discusses the alleged lack of description regarding the activities of the polypeptides of the present invention, such that agents of the present invention could not inhibit these unknown activities. Appellants respectfully traverse this rejection.

"The purpose of the written description requirement is to prevent an applicant from later asserting that he invented that which he did not; the applicant for a patent is therefore required 'to recount his invention in such detail that his future claims can be determined to be encompassed within his original creation.'" *Moba v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319 (Fed. Cir. 2003) (citing *Amgen Inc. v. Hoechst Marion Roussel Inc.*, 314 F.3d 1313, 1330 (Fed. Cir. 2003)). An accepted standard for the written description requirement is: "Although the applicant does not have to describe exactly the subject matter claimed, the description must clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1562-1563 (Fed. Cir. 1991). Written description is met if "the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Lampi*, 228 F.3d 1365, 1378 (Fed. Cir. 2000). Furthermore, the written description requirement of 35 U.S.C. § 112, first paragraph, requires that the specification "considered as a whole" describes the invention. *Reiffin v. Microsoft Corp.*, 214 F.3d 1342, 1346, 54 USPQ2d 1915, 1917 (Fed.

Cir. 2000). Appellants contend that the entire specification conveys to the skilled artisan that the methods recited in claims 78-82, 86-91 and 94 were contemplated as part of the invention.

Claims 78 and 87, the independent claims from which the remaining rejected claims depend, recite the following: "A method of treating a [patient/cell] ... comprising administering ... an effective amount of an agent that binds to a peptide or polypeptide encoded by...." (emphasis added). These claims therefore are directed to agents that bind to certain peptides and/or polypeptides. Support for these claims can be found in the specification. *See, e.g.*, page 11, lines 6-17; page 13, lines 1-3; page 44, lines 20-27; page 52, lines 12-23; page 54, lines 13-18 and page 117, lines 4-12.

For example, the specification provides examples of agents that bind to the polypeptides of the invention. Described in the specification is an antibody that was made to UC 28 (encoded by the nucleotide sequence of SEQ ID NOs: 3, 83 and 85). On page 117, lines 4 through 12 state:

> A first generation polyclonal antibody has been produced in rabbits using a KLH conjugated synthetic peptide (21 amino acids). The peptide, of sequence listed below, was chosen for antigenicity by a computer software program (DNASTAR™, Madison, WI).
>
> RKKEKVKRSQKATEFIDYSIE SEQ ID NO:56
>
> The synthetic peptide was conjugated to KLH by standard techniques and injected into two rabbits, with bleeding started at ten weeks. The antibody was peptide affinity purified and then tested in prostate cancer cell lines, and breast and prostate cancer tissue, confirming the localization of the UC 28 protein to epithelial cells, mainly on the cell membrane.

Appellants are prepared to deposit this antibody if this is deemed necessary to satisfy the written description requirement.

As an additional example, the specification recites, "The invention comprises methods of treating individuals with prostate, bladder or breast cancer by providing effective amounts of

antibodies and/or antisense DNA molecules [*i.e.*, agents] which bind to the products of the above mentioned isolated nucleic acids." Page 13, lines 1-3. Indeed, the Action concedes that agents of the present invention possess a "common ability to bind to" certain polypeptides of the present invention. The Action, page 9.

Thus, written description is provided for a species of the claimed agents, and it would be very clear to one of skill in the art that the inventors were in possession of the invention at the time of filing.

The Action further states that certain members of the genus of claimed agents are classified as inhibitors of certain peptides and polypeptides of the present invention, but that no activity has been identified for these peptides and polypeptides; as such, the Action concludes, one of ordinary skill in the art could not conclude that the Appellants were in possession of the claimed invention at the time it was filed because an inhibitor cannot be described if the activity it is allegedly inhibiting is not described. It appears that the entirety of the Action's rejection is therefore based on the contention that all agents must be inhibitors. Contrary to the Action's assertions, however, the rejected claims do not recite that all of the claimed agents must "inhibit" certain peptides and/or polypeptides: the claims instead state, as described above, that the agents must "bind to" certain peptides and polypeptides. The Action's emphasis on the alleged lack of written description with respect to inhibitors of certain peptides and polypeptides of the present invention is therefore misplaced, and cannot be used to support the written description rejection.

According to the Federal Circuit, "[i]t is well-established that a patent applicant is entitled to claim his invention generically, when he describes it sufficiently to meet the requirements of section 112." *Amgen v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016, 1027 (Fed. Cir. 1991); *see also Utter v. Hiraga*, 856 F.2d 993, 998, 6 USPQ2d

1709, 1714 (Fed. Cir. 1988) ("A specification may, within the meaning of 35 U.S.C. § 112, paragraph 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses.") As indicated above, the specification provides sufficient written description to convey to one of ordinary skill in the art that the Appellants had possession of a genus of agents that bind to certain peptides and polypeptides of the present invention.

Claims 79-82 and 86 depend from claim 78, and claims 88-91 and 94 depend from claim 87: each of these dependent claims is similarly supported by the specification and claims as originally filed. *See, e.g.*, originally-filed claims 79-82, 86-91 and 94. Consequently, each of the dependent claims fulfill the written description requirements as well.

C. Claims 78-84 and 86-94 Are Enabled

The Action maintains a rejection of claims 78-84 and 86-94 under 35 U.S.C. § 112, first paragraph, because the specification allegedly does not reasonably enable the scope of the present claims. Applicants respectfully traverse.

The general standard for enablement under § 112, first paragraph has been addressed in the case law repeatedly. For example, in *In re Wright*, 999 F.2d 1557, 27 USPQ2d 1510 (Fed. Cir. 1993), the court stated that an enabling specification teaches those skilled in the art how to make and use the claimed invention in its full scope without "undue experimentation." *Wright*, 999 F.2d at 1560. It is well-settled patent law that the first paragraph of § 112 requires nothing more than objective enablement. *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). This objective enablement may be provided through broad terminology or illustrative examples. *Id.* As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the

claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. *In re Fisher* , 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970).

(1) The Action's Arguments Based on All Agents as Inhibitors Cannot Support an Enablement Rejection

In one aspect, the Action relies on the argument presented in the written description rejection discussed above, wherein the genus of claimed agents includes inhibitors but that the function or activity of the claimed sequences is unknown—thus, the Action continues, a skilled artisan would have to perform undue experimentation to first determine such function or activity, then determine if that function or activity is related to cancer, and then design or discover an inhibitor of that function or activity. As noted above, the term "inhibits" does not appear in the present claims, but only agents that "bind to" peptides and/or polypeptides encoded by the claimed sequences. Because the present claims do not require that the agents act as inhibitors, the Action's argument cannot be used to support an enablement rejection. Furthermore, and without conceding that any inhibitors of the present invention are not enabled, "It is not a function of the claims to specifically exclude either possible inoperative substances...." *In re Dinh-Nguyen and Stenhagen,* 492 F.2d 856 (CCPA 1974); *see also In re Hradcovsky*, 214 USPQ 554 (PTO Bd. App. 1982); *Atlas Powder Co. v. E. I. du Pont de Nemours & Co.*, 588 F.Supp. 1455 (Tex. 1983).

As discussed above, the specification further provides an example of a polyclonal antibody that binds to a polypeptide (UC 28) encoded by a nucleotide sequence (SEQ ID NOs: 3, 83 and 85) of the present invention. Page 117, lines 4-12. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Failure to disclose

other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. § 112. *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987), *cert. denied*, 484 U.S. 954 (1987).

Finally, it is pointed out that effective targeting of cancer cells with a *binding agent* of the invention does not require determining the function or activity of the polypeptide, or whether the function of the polypeptide correlates with the onset of cancer, or the discovery of an inhibitor of that function activity. The Action does not assert that agents that *bind to* peptides or polypeptides of the present invention are not enabled. As such, the present claims are enabled.

(2) The Specification Does Not Fail to Teach That UC 28 Is Expressed on the Membrane of Cancer Cells

In another aspect, the Action asserts that because the specification fails to teach whether the polypeptide encoded by SEQ ID NOs: 3, 83 and 85, designated therein as UC 28, is expressed at the surface of cells, the specification therefore fails to teach whether any antibody or other inhibitor can specifically bind to and exert any inhibitory effect on those cells. Instead, the Action asserts that An *et al.* (*Cancer Res.* **60**:7014-7020, 2000, Exhibit A) provides factual evidence that UC 28 (called "UROC 28" in An *et al.*) is not expressed at the surface of cells.

In response, Appellants note that studies described in the specification on page 117, lines 10-14 indicated that the peptide encoded by SEQ ID NOs: 3, 83 and 85 was found on the cell membrane. As further evidence, Appellants submitted a declaration of Dr. Veltri (Exhibit B) in the Response to Office Action dated April 21, 2005. The declaration was made with respect to a co-pending application but is believed to be relevant here as proof that UC 28 is expressed on the membrane of cancer cells. Declaration, ¶¶ 8, 9. Therefore, one of skill in the art would expect for an agent that binds to a polypeptide encoded by SEQ ID NOs: 3, 83 and 85 to target cancer cells that overexpress these proteins.

The Action reasons that based on the following statement found on page 7017, col. 2 of An *et al.*, localization of UC 28 to the cell membrane must not have been remarkable: "UROC28 protein was localized primarily in the cytoplasm of prostate and breast cancer glandular epithelial cells." However, this statement does not make the distinction that the protein was in the cytoplasm *as opposed* to the membrane. It is perfectly consistent that the protein be in the cytoplasm but also membrane-bound—a point supported by the next statement in the An reference, which refers to nuclear localization. Page 7017, col. 2.

Furthermore, Dr. Veltri identifies amino acids 34-50 of SEQ ID NO:2 of UC 28 as a putative transmembrane domain. Declaration, ¶ 6. As such, a portion(s) of UC 28 putatively is exposed to the cell surface. Dr. Veltri's statement is supported by the abstract of the An *et al.* reference, which states: "Bioinformation analyses suggest that there is a possible transmembrane domain from amino acids aa34 to aa50...." This statement is made in a peer-reviewed article in a scientific journal, and furthermore, it adds support to the argument that the authors of this reference did not intend to distinguish cytoplasmic localization from membrane localization. Therefore, the basis for this ground of the rejection is without merit. The Action does not provide a reasonable basis for challenging the assertion in the specification that UC 28 localizes to the cell membrane, nor for challenging the assertion that at least a portion of UC 28 is expressed on the outside of the cell.

The Action tries to support the enablement rejection by asserting uncertainty with respect to this "putative" domain in combination with An *et al.*'s reference to UC 28's localization "primarily" in the cytoplasm. While the presence of the transmembrane is putative, Appellants note that in examining a patent application, the PTO is required to assume that the specification complies with the enablement provisions of Section 112 unless it has "acceptable evidence or

reasoning" to suggest otherwise. *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 369-370 (CCPA 1971). As discussed, localization of UC 28 "primarily" in the cytoplasm does not exclude its localization to the membrane. The presence of a putative transmembrane domain, in combination with Dr. Veltri's statement that UC 28 is localized to the cell membrane, together establish that UC 28 is localized to the cell membrane, thereby overcoming the enablement rejection.

(3) The Specification Is Enabling for the Design of Chemotherapeutic Agents

With respect to claims 84 and 93, the Action asserts that the degree of unpredictability and extreme complexity in the art of anticancer drug discovery is such that a chemotherapeutic agent of the present invention cannot be recognized or made by routine experimentation alone. Appellants assert that use of anticancer agents of the invention does not require undue experimentation. For example, in certain embodiments of the invention, binding agents may be conjugated to radionuclides or to chemotherapeutic agents. Use of radionuclides and chemotherapeutic agents is well known in the art, and both radionuclides and chemotherapeutics are widely used in the treatment of cancer. Conjugation of radionuclides and/or chemotherapeutics to agents of the invention may increase their efficacy or reduce toxicity to healthy tissue. Thus, there would be no requirement for the kind of protracted analyses that the Action indicates would be necessary in order to practice the invention.

Moreover, as has been determined by the courts, the scope of the enablement must only bear a "reasonable correlation" to the scope of the claims. *In re Fisher* , 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Even if experiments are necessary, a considerable amount of routine experimentation is permissible; the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Wands*, 858

F.2d 737, 8 USPQ2d 1404 (Fed. Cir. 1985); *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd. sub nom. Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985). "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine." *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)). Thus, even if extensive experimentation is necessary to identify chemotherapeutic agents of the present invention, such experimentation does not mean the specification fails to enable the present claims.

In fact, binding agents that target cancer cells, such as those of the current invention have been used in clinical trials. Carroll, 2004 (Exhibit C) reports use of a yttrium-90 labeled monoclonal antibody targets a membrane protein on prostate cancer cells. Results from this study indicated that the antibodies labeled with the radionuclide had "[a]cceptable toxicity, excellent targeting of known sites of PC metastases, and biologic activity" in patients. Thus, Carroll indicates that agents for the treatment of cancer such as those of the invention are known to be effective for cancer therapy, and even for the treatment of solid tumors. As detailed above the specification provides enabling written description that would allow a person of normal skill in the art to apply the invention for the treatment of cancer without undue experimentation.

(4) The Specification Demonstrates a Correlation Between the Level of mRNA Expression and the Level of Protein Expression in Cancer Cells

The Action further indicates that the specification teaches that mRNAs corresponding to the sequences of the invention are overexpressed in cancer cells; however, the Action then states that it does not teach that the polypeptide encoded by these RNAs are overexpressed *per se* and therefore, a method for treating cancer by targeting cells overexpressing these polypeptides lacks

enablement. Appellants respectfully traverse, because it is demonstrated in the specification that, for instance, UC 28 mRNA is overexpressed in breast cancer cells (FIG. 15), 4 out 5 bladder cancer cell lines (FIG. 16) and is hormone inducible in prostate in a prostate cancer cell line (FIG. 17). The Action concedes that the specification teaches overexpression in breast and prostate cancer cells. The Action, page 21. Additionally, U.S. Patent Application Serial No. 08/692,787 (now U.S. Patent No. 5,886,284), incorporated by reference by the present specification at page 1, lines 6-8, describes the overexpression of UC 28 in prostate cells (*see, e.g.*, Figure 3). While overexpression of the mRNAs in one cell line might result from random mutation during cancer development, overexpression in a wide range of cells would suggest to one of skill in the art that overexpression of the polypeptide was in fact advantageous to the cancer cell. Thus, the demonstration that a variety of cells overexpress the sequences of the invention implicitly indicates corresponding polypeptide overexpression.

Therefore, it is clear to one of skill in the art that the specification does teach that UC 28 protein is overexpressed in the cancer cells recited in the claims, thus enabling a method of treating cancer that targets cells expressing UC 28.

For the foregoing reasons, Appellants respectfully request this rejection be withdrawn.

D. Claims 78-82, 86-91 and 94 Are Not Indefinite

The Action rejects claims 78-82, 86-91 and 94 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Action contends that the phrase "effective amount" is indefinite for allegedly failing to state the function that is to be achieved. More specifically, the Action states that it cannot be determined if the claim requires the "effective

amount" of an agent to be sufficient to effectively inhibit the polypeptide, or to effectively treat cancer in a patient, or both. Appellants respectfully traverse.

The standard of precision regarding indefiniteness is "whether one skilled in the art would understand the bounds of the claim when read in light of the specification.... If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more." *Miles Laboratory, Inc. v. Shandon Inc.*, 27 USPQ2d 112, 1126 (Fed. Cir. 1993). *See also Union Pacific Resources Co. v. Chesapeake Energy Corp.*, 57 USPQ2d 1293 (Fed. Cir. 2001) and MPEP § 2173.02. When read in light of the specification, the phrase "effective amount" is definite and satisfies all of the requirements of 35 U.S.C. § 112, second paragraph.

The phrase "effective amount" is found in independent claims 78 and 87, which recite: "A method of treating [a patient with breast cancer, bladder cancer or prostate cancer/a breast cancer, bladder cancer or prostate cancer cell] comprising administering to the [patient/cell] an effective amount of an agent that binds to a peptide or polypeptide encoded by...." As discussed above, Appellants note that the claims do not recite the term "inhibit" with respect to administration of the claimed agents, but instead that "an effective amount of an agent that *binds to* a peptide or polypeptide" is administered (emphasis added). Thus, to the extent the Action's argument is based upon any required inhibitory activity of the claimed agents, the argument cannot support an indefiniteness rejection.

The meaning of the phrase "effective amount" is described in the specification: "An effective amount of the therapeutic composition is determined based on the intended goal." Specification, page 83, lines 29-30. It is clear to one of ordinary skill in the art that the goal of the rejected claims is to treat either: (a) a patient with breast cancer, bladder cancer or prostate

cancer, or (b) a breast cancer, bladder cancer or prostate cancer cell, such that the amount of the agent administered binds to a peptide or polypeptide encoded by a claimed sequence. Accordingly, an "effective amount" of an administered agent is one that results in treatment of a patient or cell via binding to a peptide or polypeptide encoded by a claimed sequence. Therefore, this phrase is not indefinite, and one of ordinary skill in the art would understand the meaning and use of this phrase in the claims when read in light of the specification.

Further, one of ordinary skill in the art would be able to determine from the specification what an effective amount is. For example, page 83, line 29 through page 84, line 11, give guidance to a skilled artisan as to how to determine an effective amount, including exemplary unit dosages that may be administered. Such descriptive support renders the claims not indefinite. *See, e.g., Ex part Skuballa*, 12 USPQ2d 1570 (Bd. Pat. App. & Inter. 1989) and MPEP § 2173.05(c).

The rejection of the phrase "effective amount" as being indefinite is therefore improper and should be withdrawn.

E. Conclusion

For the above-argued reasons, Appellants respectfully request that the rejection of claims 78-84 and 86-94 be reversed. Appellants have provided arguments that overcome the pending rejections. Appellants respectfully submit that the Examiner's conclusion that the claims should be rejected is legally and factually unsupported. It is therefore again requested that the Board overturn the Examiner's rejection.

Please date stamp and return the enclosed postcard to evidence receipt of this document.

Respectfully submitted,

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Date: October 16, 2006

VIII. CLAIMS APPENDIX

78. A method of treating a patient with breast cancer, bladder cancer or prostate cancer comprising administering to the patient an effective amount of an agent that binds to a peptide or polypeptide encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:83 or SEQ ID NO:85, or a fragment thereof.

79. The method of claim 78, wherein the agent is an antibody.

80. The method of claim 79, wherein the antibody is specific to a polypeptide encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:83 or SEQ ID NO:85 or a fragment thereof.

81. The method of claim 80, wherein the antibody is a monoclonal antibody.

82. The method of claim 80 wherein the antibody is a polyclonal antibody.

83. The method of claim 80, wherein the antibody is conjugated to a radionuclide.

84. The method of claim 80, wherein the antibody is linked to a chemotherapeutic agent.

86. The method of claim 78, wherein the agent binds to a polypeptide encoded by SEQ ID NO: 3, SEQ ID NO: 83 or SEQ ID NO: 85 or a fragment thereof.

87. A method of treating a breast cancer, bladder cancer or prostate cancer cell comprising administering to the cell an effective amount of an agent that binds to a peptide or polypeptide encoded by SEQ ID NO:3, SEQ ID NO:83 or SEQ ID NO:85, or a fragment thereof.

88. The method of claim 87, wherein the agent is an antibody.

89. The method of claim 88, wherein the antibody is specific to a polypeptide encoded by SEQ ID NO:3, SEQ ID NO:83 or SEQ ID NO:85 or a fragment thereof.

90. The method of claim 88, wherein the antibody is a monoclonal antibody.

91. The method of claim 88, wherein the antibody is a polyclonal antibody.

92. The method of claim 88, wherein the antibody is conjugated to a radionuclide.

93. The method of claim 88, wherein the antibody is linked to a chemotherapeutic agent.

94. The method of claim 87, wherein the cell is in a patient.

IX. EVIDENCE APPENDIX

Exhibit A: An *et al.*, *Cancer Res* **60**:7014-7020, 2000.

Exhibit B: Declaration of Dr. Robert W. Veltri, Ph.D.

Exhibit C: Carroll, 2004, Commentary to "Phase I trial of yttrium-90-labeled anti-prostate specific membrane antigen monocolonal antibody J591 for androgen-independent prostate cancer," *J. Clin. Oncol* **22**:2522-2531, 2004.

X. RELATED PROCEEDINGS APPENDIX

[NONE]

EXHIBIT A

[CANCER RESEARCH 60, 7014–7020, December 15, 2000]

Cloning and Characterization of *UROC28*, a Novel Gene Overexpressed in Prostate, Breast, and Bladder Cancers[1]

Gang An, Angela Y. Ng, C. S. Reddy Meka, Guizhen Luo, Steven P. Bright, Lisa Cazares, George L. Wright, Jr., and Robert W. Veltri[2]

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ABSTRACT

A novel gene, designated *UROC28*, was identified by an agarose gel-based differential display technique, and it was found to be up-regulated in prostate, breast, and bladder cancer. Expression of *UROC28* was also up-regulated in prostate cancer cells in the presence of androgens as demonstrated by relative quantitative reverse transcription-PCR. The elevated expression of this gene was observed to increase in surgically removed tissues concomitantly with rising Gleason grade and was most elevated in metastatic tissue. UROC28 protein was detected in serum by Western slot blot analyses, and a significant higher UROC28 protein level was found in sera of prostate cancer individuals compared with normal individuals and individuals with nonmalignant prostatic hyperplasia. Northern analyses in normal tissues showed that the UROC28 cDNA hybridizes to two mRNAs at about 2.1 and 2.5 kb. Nucleic acid sequence analyses indicated that these two alternatively spliced mRNA variants differ only at the 3′ untranslated region. These two mRNAs encode the same protein with 135 amino acids. Bioinformation analyses suggest that there is a possible transmembrane domain from amino acid aa34 to aa50, three protein kinase-C phosphorylation sites at aa62 (SQK), aa89 (TMK), and aa94 (SMK), and one myristylation site at aa118 (GLECCL). Genomic Southern hybridization and chromosomal mapping demonstrated that *UROC28* is encoded by a single copy of gene at chromosome *6q23–24*. *In situ* hybridization and immunohistochemistry experiments further confirmed up-regulation of this gene in prostate and breast cancers with the expression localizing to the glandular epithelium. This gene did not demonstrate increased expression in lung and colon cancer tissues.

INTRODUCTION

Prostate cancer is the most common malignancy among men in the United States, affecting over 179,300 men and resulting in about 37,000 deaths in 1999 (1). A small percentage (about 10–15%) of newly diagnosed cancers are actually metastatic at the time of diagnosis (2, 3). Approximately 30% of men who are treated for localized disease will recur and a subset of these men will progress to androgen-independent metastatic prostate cancer (1–3). The mechanism(s) for disease progression and development of the androgen-independent state remains poorly understood. It is not clear why some patients with prostate cancer progress so quickly and others do not. It is possible that multiple genetic and/or epigenetic factors contribute to the biological heterogeneity of prostate cancer and the variability in the rate of progression and disease-specific mortality (4, 5). Identification of genetic and epigenetic factors that may play important roles in prostate cancer progression and metastasis is of great significance to prostate cancer management.

Like many other cancers, the development of prostate cancer is a multistage process involving initiation, progression, invasion, and metastasis (6, 7). Studies have demonstrated that transformation of a normal cell to a fully malignant cell requires a series of genetic changes including mutations of DNA and changes of gene expression at the RNA and protein levels (8, 9). Recently, several laboratories including ours have been actively involved in identifying genes associated with prostate cancer progression and metastasis. These efforts have resulted in the discovery of several genes involved in different biochemical pathways related to the pathogenesis of prostate cancer. Examples of genes identified include but are not limited to HER2/*neu* (10), *prostate-specific transglutaminase* (*pTGase*; Refs. 11, 12), *PSMA* (13), *caveolin* (14), *PTEN* (15), *PSCA* (16), *POV1* (17), *NKX3.1* (18), and *ETS-2* (19).

In search of potential new gene markers for prostate cancer, we have applied a modified, agarose gel-based differential display method (20, 21) to isolate genes differentially expressed among normal prostate, prostate cancer, and metastatic prostate cancer tissues. We report here the cloning and characterization of a novel gene, *UROC28*, that is overexpressed in prostate, breast, and bladder cancer. The full cDNA sequence, chromosomal localization of the gene, the development of a specific polyclonal antibody, and detection of the UROC28 protein in serum are also described. The data indicate a correlation between overexpression and the pathogenic determinants of prostate cancer, which may support its eventual application in diagnosis and treatment of prostate cancer.

MATERIALS AND METHODS

Frozen Tissue Samples and RNA Isolation. Frozen tissues used in the experiments were obtained from the CHTN[3] (Birmingham, AL), the Virginia Prostate Center Tissue Bank at Eastern Virginia Medical School, (Norfolk, VA), and the Department of Urology, University of Washington, (Seattle, WA). Pathological reports were provided by the organizations for tissue specimens. The specimens were quick frozen in liquid nitrogen immediately after surgery and stored at −70°C until processed for RNA isolation. Total RNAs were isolated from the specimens as described previously (22). Total RNAs (10 μg) from each tissue were treated with 5 units of RNase-free DNase I (Life Technologies) in the presence of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$ and 20 units of RNase inhibitor (Boehringer Mannheim). After extraction with phenol/chloroform and ethanol precipitation, the RNA was redissolved in diethylpyrocarbonate-treated H_2O.

Differential Display. A modified, agarose gel-based differential display (20, 21) was used to identify genes differentially expressed in prostate cancer. RNA (10 μg) from each tissue was treated with RNase-free DNase I as described above. Five μg from each of the RNA samples was reverse transcribed into cDNA using random hexamers and Moloney-murine leukemia virus (M-MLV) reverse transcriptase (Life Technologies) following manufacturer's instructions. The reaction mixture contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 500 μM dNTP, 2 μM random hexamers and 400 units of M-MLV reverse transcriptase. PCR was performed with one arbitrary 10mer. The primer used for identifying UROC28 was 5′-TGGAG-GTTGT-3′. PCR conditions were as follows: 1× PCR buffer [50 μM dNTPs,

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[3] The abbreviations used are: CHTN, Cooperative Human Tissue Network; FISH, fluorescent *in situ* hybridization; NMPH nonmalignant prostatic hyperplasia; NEM, no evidence of malignancy; RT-PCR, reverse transcription-PCR; DHT, dihydrotestosterone; DAPI, 4′,6-diamidino-2-phenylindole; HRP, horseradish peroxidase; DAB, 3,3′-diaminobenzadine; CV, coefficient of variation; BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; CaP, prostate cancer.

0.2 μM arbitrary primer(s), 1/20 volume (1 μl) of the cDNA, 1 unit of Taq DNA polymerase (Life Technologies)] in a final 20-μl mixture. The amplification parameters included 40 cycles of reaction with 30 s denaturing at 94°C, 1 min 30 s annealing at 38°C, and 1 min extension at 72°C. A final extension at 72°C was performed for 15 min. The PCR products were then separated on a 2% agarose gel with 0.5 μg/ml ethidium bromide, and positive bands were identified, excised, purified by Qiaex resin (Qiagen), and cloned into plasmid by TA cloning (Promega). The differential expression of positive bands was confirmed by relative quantitative RT-PCR (10, 12). A gene, designated *UROC28*, was found up-regulated in prostate cancer.

Full-Length cDNA Cloning and Sequencing. A human prostate cDNA library constructed in λgt10 vector was purchased from Clontech and used for full-length cDNA cloning of UROC28. The method of Benton and Davis (23) was followed for cDNA library screening. A 0.6-Kb UROC28 cDNA fragment was labeled with ^{32}P using High Prime system (Boeringer Mannheim). Two to three rounds of rescreening were carried out to obtain a pure positive clone. Both strands of the isolated cDNA clones were sequenced by the dideoxynucleotide-chain termination method (24) using a primer walking strategy.

Chromosomal Mapping. The procedure for FISH chromosomal localization of UROC28 was performed according to Heng *et al.* (25). Briefly, lymphocytes were cultured in a MEM supplemented with 10% FCS and phytohemagglutinin at 37°C for 68–72 h. The lymphocyte cultures were then treated with bromodeoxyuridine (0.18 mg/ml; Sigma) to synchronize the cell population. The cells were then washed with serum-free medium to release the block and recultured at 37°C for 6 h in MEM with thymidine (2.5 μg/ml; Sigma). Cells were harvested and slides were made by using standard procedures. Slides were baked at 55°C for 1 h. After RNase treatment, the slides were denatured in 70% formamide in 2x SSC for 2 min at 70°C followed by ethanol dehydration. The 0.6-Kb UROC28 cDNA probe was biotinylated with dATP for 1 h at 15°C using BioNick labeling kit (Life Technologies). The labeled probe was denatured at 75°C for 5 min in a hybridization solution containing 50% formamide, 10% dextran sulfate, and human cot I DNA. The denatured probe was loaded onto the slides and subjected to overnight hybridization. Slides were then washed, detected, and amplified. FISH signals and DAPI banding pattern were recorded separately by taking photographs, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI-banded chromosomes.

RT-PCR and Northern Hybridization. Five μg of the DNA-free total RNA was reverse transcribed into cDNA using random hexamers and M-MLV reverse transcriptase (Life Technologies) following manufacturer's instructions. The reaction mixture contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 500 μM dNTP, 2 μM random hexamers, and 400 units M-MLV reverse transcriptase. The reaction was incubated at 22°C for 10 min, then at 37°C for 50 min. The synthesized cDNA was used for PCR. The primers used for PCR and their sequences are as follows: UROC28-P1, 5′-GCT TCA GGG TGG TCC AAT TAG AGT T-3′; and UROC28-P2: 5′ TCC AAC AAC GAC ACA TTC AGG AGT T 3′.

The primers amplified a 446-bp PCR product from the human UROC28 cDNA. The relative abundance of UROC28 in the tissues was studied by a relative quantitative RT-PCR using β-actin as a control. The PCR mixture contained 2 μl of cDNA, 10 mM Tris-HCl (pH 9.3), 50 mM KCl, 3 mM $MgCl_2$, 200 μM dNTP, 1.25 units of Taq DNA polymerase (Life Technologies), and 200 nM of sense and antisense primers in a total of 50-μl reaction. The amplification was performed in a thermal cycler (MJ Research), which included 1-min denaturing at 94°C, 1-min annealing at 56°C, and 1-min extension at 72°C. The PCR was run for 35 cycles for UROC28 and 22 cycles for β-actin. The PCR products were run on a 1.2% agarose gel with ethidium bromide. The UROC28 bands were quantitated by an IS-1000 image analyses system (Alpha Innotech) and normalized with that of β-actin control. All of the normalized values are presented as the mean ± SD.

The filter for Northern hybridization was purchased from ClonTech and was hybridized to the 446-bp [^{32}P]-labeled UROC28 cDNA probe. The ClonTech Multiple Tissue Northern blots contained 2 μg of oligo(dT)-purified mRNA from different specific normal human tissues. Hybridization, washing, and X-ray film exposure were performed as described previously (26). After stripping, the same filter was hybridized to the β-actin probe.

Cell Culture and DHT Treatment. LnCaP cell line was obtained from American Type Culture Collection (Rockville, MD). The cells were cultured in RPMI 1640 with 10% charcoal-stripped serum for 48 h first, then incubated with RPMI 1640 with 10% charcoal-stripped serum in the presence of 0, 0.1, 1, 10, or 100 nM DHT for 24 h, respectively. RNA was isolated and subjected to RT-PCR analyses as described above.

***In Situ* Hybridization.** UROC28-specific antisense nucleotide probe (5′-TCT TAA CTC GGG GCA TTT GGT CTT C-3′) and the corresponding sense probe were synthesized and labeled with biotin at the 3′ ends. Sense probe was used as the negative control. Hybridization was performed on formalin-fixed paraffin sections using a MicroProbe System (Fisher Scientific). Paraffin-embedded and formalin-fixed tissues and their corresponding pathological diagnoses were obtained from the CHTN and Department of Pathology, Johns Hopkins Medical Institutions (Baltimore, MD). All of the reagents and diluents used were obtained from Research Genetics (Huntsville, AL). Briefly, paraffin sections in 5-μm thickness were deparaffinized using Auto Dewax solution and dehydrated with alcohol. Sections were then treated with Auto Blocker to block any endogenous peroxidase activity. A pretreatment with pepsin solution for 3 min at 105°C was followed by probe (100-ng/ml) incubation. Hybridization was carried out at 105°C for 5 min and then at 45°C for one h. Sections were then washed with PostHyb Wash solution for 5 min at 45°C before incubation with strepavidin-HRP for 5 min at 50°C and followed by two changes of DAB substrate incubation for 5 min (50°C) each. Sections were counterstained with hematoxylin for 1 min before dehydration and mounting. Polydeoxythymidylic acid hybridization control was performed in parallel to confer the general mRNA integrity in the paraffin sections.

Immunohistochemistry. Anti-UROC28 rabbit polyclonal antibody was produced using a synthetic peptide as the immunogen, which corresponds to the predicted amino acid 54 to 74 of UROC28 (Research Genetics, Huntsville, AL). The antibody was peptide-affinity purified, and immunohistochemistry was performed on formalin-fixed paraffin sections using a MicroProbe System (Fisher Scientific). After dewaxing and dehydration, sections were microwaved with citrate buffer (pH 6.0) for two time for 5 min each. The sections were washed with deionized water and PBS (pH 7.4), then incubated with 0.5% Triton X-100 and 0.5% milk in PBS for 5 min at room temperature. The sections were blocked with 5% milk in PBS containing 0.1% Triton X-100 for 20 min, then incubated with the rabbit polyclonal antibody diluted 1:1000 with PBS containing 0.5% milk and 0.1% Triton X-100 at 4°C overnight in a humidified chamber. After washing three times with PBS/0.1% Triton X-100, the sections were sequentially incubated for 20 min each with SuperSensitive biotinylated MultiLink secondary antibody, streptavidin-alkaline phosphatase (Biogenex), and freshly prepared Vector Red chromogen substrate (Vector Laboratories). The sections were counterstained with hematoxylin for one min followed by dehydration and mounting. Rabbit preimmune serum was used as the negative control. Sequential tissue sections used for UROC28 *in situ* hybridization were used for immunohistochemistry.

Western Slot Blot. Serum specimens from 18 normal individuals, 15 biopsy-confirmed patients with NEM, and 14 CaP patients with the clinical cancer stage ranging from T_{1a} to T_4 and Gleason scores ranging from 3 to 7 (average, 6), were studied. The normal sera were residual sera obtained from healthy male blood donors from the Oklahoma Blood Institute (OBI) of Oklahoma City, OK, and donors' confidentiality is strictly held under guidelines at OBI. The patients' sera (NEM and PCa) were residual samples from Institutional Review Board-approved cancer biomarker studies previously conducted at UroCor with collaborators from Johns Hopkins Medical Institutions and University of Michigan Cancer Center. The clinical diagnoses of these patients were provided by our urologist collaborators. All of the patients' personal identifications were kept confidential and remain unknown to UroCor staff. Each serum specimen was assayed in duplicate, and the CV was recorded. Twenty μl of serum from each normal or patient test sample was diluted to 100 μl with Tris-buffered solution (TBS) and blotted in duplicate onto nitrocellulose filter using a slot blot apparatus from Bio-Rad Laboratories (Hercules, CA). After blocking with 5% nonfat milk, the filter was incubated with the polyclonal UROC28 antibody described in "Immunohistochemistry" in this section in 1:500 dilution overnight at 4°C. The filter was then washed and incubated with 1:1000 alkaline phosphate-conjugated goat antirabbit immunoglobulins (DAKO, Carpinteria, CA) for 1 h., washed, and then incubated with 5-bromo-chloro-3-indolylphosphate petoluidine salt/nitroblue tetrazolium chloride chromogen/substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The alkaline phosphate signal intensity of the UROC28 protein-antibody immune complexes bands were quantitated by an IS-1000 image analyses

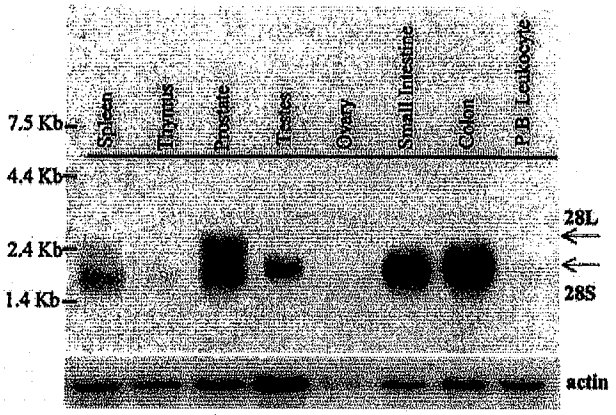

Fig. 1. Expression of *UROC28* mRNA in various human tissues. Northern hybridization was performed on human Multiple Tissue Blot II from Clontech (Palo Alto, CA). There are eight tissues with two μg of mRNA per lane on the blot as indicated. The blot was hybridized with ^{32}P-labeled DNA probe prepared from the original 0.6-kb UROC28 fragment. *Arrows*, the two mRNA variants (UROC28L and UROC28S for the long and short transcripts, respectively) detected by the probe. The same filter was striped and hybridized with *β-actin* probe.

system (Alpha Innotech). The positive signal intensity was quantitated using relative absorbance units based on optical density. The average of duplicate test samples was used for analysis. The serially diluted synthetic UROC28 peptide was used as the positive control standard for each slot blot immunoassay. Any patient's sera with an absorbance value outside the linear range of detection from the peptide standard curve were diluted appropriately and reassayed when necessary. Statistical analyses were performed using the Stata v5.0 statistical software program (STATA Corp., College Station, TX).

RESULTS

Cloning of *UROC28* cDNA. A modified agarose gel-based mRNA differential display method (21) was used to identify genes differentially expressed in prostate cancer tissue. UROC28 was identified as one of several genes overexpressed in prostate cancer by comparing display band patterns between normal prostate and prostate cancer. The original identified UROC28 cDNA fragment was determined to be ~0.6 kb. After cloning into pGEM-T plasmid vector, the fragment was then fully sequenced. A GenBank search indicated that the UROC28 fragment did not match any known genes in the database. The human tissue specificity and the mRNA transcript size of UROC28 were evaluated using Northern blot analysis. Northern hybridization of the UROC28 fragment to mRNAs from eight different organs showed a major 2-kb band in colon, prostate, small intestine, testes, and spleen, the expression was minimal in thymus, ovary, and peripheral blood leukocytes. An additional band at 2.4 kb was seen in prostate, and also in spleen but with less intensity, which indicated the possibility of two alternative splicing variants for this gene (Fig. 1).

The full-length cDNAs of the two alternative splicing mRNA variants were cloned by cDNA library screening and sequencing. As shown in Fig. 2, the two mRNA variants are identical for 1.96 kb of their 5′ sequences, including the 5′-untranslated region, the complete open reading frame, and part of the 3′ untranslated region. They differ only at the end of the 3′ untranslated region starting at 1960 bp. Both mRNAs have polyadenylic acid tails and the predicted polyadenylation sites. The two mRNAs have the same open reading frame, encoding a protein of 135 amino acids (Fig. 2). A strong Kozak consensus sequence is found preceding the ATG initiation codon. Bioinformatics analyses indicate that there is a possible transmembrane domain from aa34 to aa50, three PKC phosphorylation sites at aa62 (SQK), aa89 (TMK), and aa94 (SMK), and one myristylation site at aa118(GLECCL). *In vitro* translation experiments using rabbit reticulocyte lysate with both cDNA variants generated a single M_r 17,000 protein product, which is the predicted size from the open reading frame (data not shown).

Differential Expression of *UROC28* in Prostate Cancer. The differential expression of *UROC28* gene in prostate cancer was first confirmed using primers unique to each variant in a relative quantitative RT-PCR experiment. RT-PCR was performed on five to six frozen tissues each from normal prostate, BPH, and prostate cancer at different Gleason scores as well as from metastatic prostate cancer specimens. As shown in Fig. 3, the expression of both UROC28 variants is low in all of the normal and BPH tissues and is up-regulated in all of the prostate cancer and metastatic cancer tissues tested in 35 PCR cycles.

The differential expression of both UROC28 transcript variants in prostate cancer was further investigated in an expanded panel of frozen tissues. RNA was isolated from 8 normal prostate tissues, 20 BPH tissues, 28 prostate cancer tissues with different Gleason scores, and 7 metastatic prostate cancer tissues. RT-PCR was performed using primers unique to both variants, and the expression levels were normalized with that of *β-actin*. As shown in Fig. 4, both variants were similarly up-regulated in prostate cancer and metastatic prostate cancer tissues. A low level of expression for both variants was observed in normal tissues and NMPH tissues. Both UROC28 transcript variants were up-regulated 3- to 4-fold in prostate cancer with varying Gleason scores, and 4- to 6-fold in metastatic prostate cancer tissues.

Expression of UROC28 mRNA and Protein in Glandular Epithelial Cells of Prostate and Breast Cancers. *In situ* hybridization with biotin-labeled UROC28-specific oligonucleotide probe with streptavidin-HRP and DAB substrate demonstrated that UROC28 mRNA was preferentially expressed in prostate and breast glandular or ductal epithelial cells (Fig. 5, *E–H*). The reporter HRP enzyme

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A   99 ATG AGG GCC TTC TTA AGG AAC CAG AAA TAT GAG GAT ATG CAC AAT ATT ATT CAC ATT TTA CAG ATC AGA
         M   R   A   F   L   R   N   Q   K   Y   E   D   M   H   N   I   I   H   I   L   Q   I   R
  168 AAA TTG AGG CAC AGA TTA AGT AAC TTC CCA AGG CTA CCA GGC ATT CTA GCT CCA GAA ACT GTG CTC TTA
         K   L   R   H   R   L   S   N   F   P   R   L   P   G   I   L   A   P   E   T   V   L   L
  237 CCA TTC TGC TAC AAG GTA TTT CGA AAA AAA GAA AAA GTA AAA AGA AGT CAA AAG GCA ACA GAG TTC ATT
         P   F   C   Y   K   V   F   R   K   K   E   K   V   K   R   S   Q   K   A   T   E   F   I
  306 GAT TAT TCC ATA GAA CAG TCA CAC CAT GCA ATT CTC ACA CCC TTG CAG ACA CAC TTG ACC ATG AAA GGT
         D   Y   S   I   E   Q   S   H   H   A   I   L   T   P   L   Q   T   H   L   T   M   K   G
  375 TCC TCA ATG AAA TGT TCC TCA TTA TCT TCA GAA GCC ATA TTA TTC ACA TTG ACT TTG CAG TTA ACT CAG
         S   S   M   K   C   S   S   L   S   S   E   A   I   L   F   T   L   T   L   Q   L   T   Q
  444 ACC CTA GGT CTG GAA TGC TGT CTT CTC TAC TTA TCC AAA ACT ATA CAT CCA CAG ATC ATA TAA 506
         T   L   G   L   E   C   C   L   L   Y   L   S   K   T   I   H   P   Q   I   I   *

B  1961  ATTAAAACAT TACCGAGAGG GGACTCAAAC AGTCTTTCTT CCTTTGTCGT GTTTCTTGCT CCCAGACCAA GGCACTGACG
   2041  ACAGTACTGA TACATAATTT AAAAGCACAC TCCCTTCCAC TTTGGTAATA CCAGAACTCT AATTGGACCA CCCTGAAGCT
   2121  TAGGACTACC AGCCATACAA ATAGTAAACT CTGTCCACGA TTCACTCATC TGTGTATTTT CTATAGATGT TTACTAGGCG
   2201  TTTGTTATAT AAAAATACCC CGGCCAGGCA CGGTGGCTCA CGCCTGTAAT CCCAGCACTT TGGGAGGTGG GTGGATCACC
   2281  TGAGGTCGGG AGTTCGAGAC CAGCCTGACC AGCATGGTGG AACCCCCATC TCTACTAAAA ACACAAAAAA TTAGCCGGGC
   2361  GTGGTGGCAC ATGCCTGTAA TCCCAGCTAC TCAGGAGGCT GAGGCGGAGA ATTGCTTGAA CCCGGAAGGT GGAGGTTGTT
   2441  GCGGTGAGCT GAGATTGCAC TATTGCACTC CAGCCTGGGC AACAGGAGTA AAACTCCCCC CCACCCAAAA AA  2512

C  1961  TGCACGACAA CACATAAAAA AAGAGAGAAG AATCAAATAG ACACAATAAA AAATGATAAA GGGGATATCA CCACCGATCC
   2041  CACAGAAATA AAAACTACCA TCAGAGAATA CTACAAACAC CTCTACGCAA AAAAAAAAAA  2100
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Fig. 2. Nucleotide sequences and predicted amino acid sequence of the two UROC28 transcript variants. The two variants share the same 1960-bp 5′ sequence and the open reading frame. *Underlined*, the polyadenylation sites of the two variants. *A*, open reading frame and deduced amino acid sequence of both of the UROC28 transcripts. *B*, the different 3′ end nucleotide sequence of the UROC28 long transcript. *C*, the different 3′ end nucleotide sequence of the UROC28 short transcript. The full sequences of both forms have been deposited in the GenBank database [accession no. AF189269 (long transcript) and AF189270 (short transcript)].

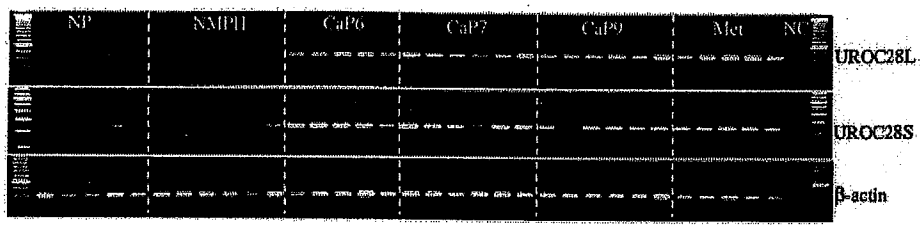

Fig. 3. Confirmation of UROC28 differential expression by relative quantitative RT-PCR. The results of RT-PCR for UROC28 long and short transcripts, and *β-actin* control are shown. *NP*; normal prostate tissues; *CaP6*, prostate cancer at Gleason score 6; *CaP7*, prostate cancer at Gleason score 7; *CaP9*, prostate cancer at Gleason score 9; *Met*, metastatic prostate cancer; *NC*, negative control.

reacted with DAB and yielded a brown precipitate reflecting the level of hybridized signals. Paraffin sections from prostate cancer with different Gleason scores ranging from 6 to 9 were analyzed for the cellular localization and relative expression level of UROC28 mRNA. Elevated, yet heterogeneous, UROC28 mRNA hybridization signal was observed in prostate cancer glandular epithelium. Fig. 5*F* showed the elevated UROC28 mRNA signal in Gleason score 8 prostate cancer glandular epithelia as compared with the adjacent or distant prostate acini without evidence of malignancy (Fig. 5*E*). UROC28 mRNA was expressed primarily in the basal cells of the benign prostate acini (Fig. 5*E*). Polydeoxythymidylic acid hybridization was performed on these prostate sections as a positive control for the integrity of the mRNA in the tissue sections. *In situ* hybridization analysis also showed the up-regulation of UROC28 mRNA in intraductal breast cancer cells (Fig. 5*H*) as compared with breast tissue without pathological evidence of malignancy (Fig. 5*G*). These observations are in concordance with the PCR results described below.

Rabbit polyclonal anti-UC 28 antibody immunostaining also demonstrated the expression of UROC28 protein in prostate glandular epithelial cells. This polyclonal antibody was raised against the putative cytoplasmic region of the UROC28 protein. Positive immunostaining is indicated (Fig. 5) by the *red precipitate* from alkaline phosphatase reporter enzyme and the substrate. Serial tissue sections used in the *in situ* localization of UROC28 mRNA were analyzed for the expression of UROC28 protein. Similar to the *in situ* hybridization findings, increased level of UROC28 protein was observed in prostate cancer glandular epithelial cells (Fig. 5*B*) as compared with the prostate glandular epithelium without pathological evidence of malignancy (Fig. 5*A*). Elevated UROC28 protein was also noted in ductal epithelial cells in breast cancer sections (Fig. 5*D*) as compared with the breast counterpart without pathological evidence of malignancy (Fig. 5*C*). UROC28 protein was localized primarily in the cytoplasm of prostate and breast cancer glandular epithelial cells (Fig. 5*A–D*). However, distinct nuclear localization of UROC28 protein was also noted in prostate cancer glandular epithelia (Fig. 5*B*).

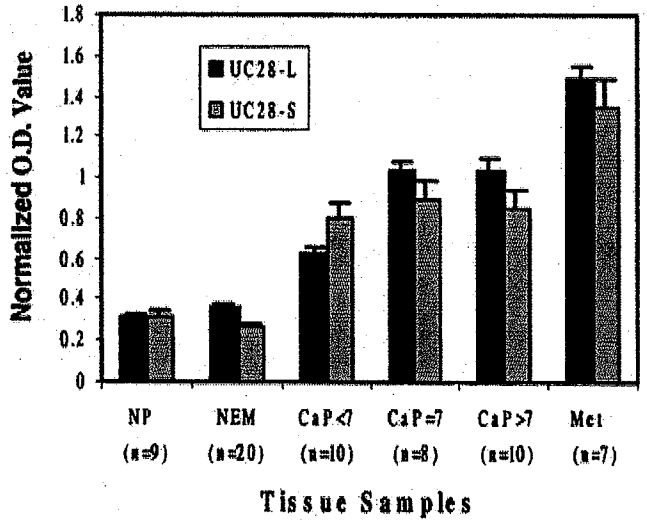

Fig. 4. Expression of UROC28 in prostate cancer with different Gleason scores and metastatic prostate cancer. RT-PCR was performed with primers specific to each UROC28 variant (35 PCR cycles) and β-actin (22 PCR cycles). The RT-PCR bands were quantitated by densitometric analyses, and the absorbance of each UROC28 variant was normalized with that of β-actin. *NP*, normal prostate; *CaP*, prostate cancer (Gleason score); *Met*, metastatic prostate cancer; *n*, the number of tissues used in each sample group. All of the normalized values are presented as the mean ± SD.

Expression of UROC28 Protein in Serum. A Western slot blot protocol was used to investigate whether UROC28 protein is present in sera of normal and prostate cancer individuals. As shown in Fig. 6, the mean serum UROC28 protein level in individuals with prostate cancer is significantly higher than both normal and NEM individuals at 95% confidence interval ($P < 0.001$). Student's t test analysis demonstrated that the mean serum UROC28 protein level between BPH and prostate cancer was significantly different ($P = 0.0003$). Also, the mean URCO28 levels between normal *versus* NEM and normal *versus* prostate cancer were both significantly different with a $P < 0.0001$. These Western slot blot assays performed reproducibly demonstrating an interassay CV of 11% and an intraassay CV of 8%.

Expression of UROC28 in Other Cancer Tissues. To investigate whether *UROC28* gene is also differentially expressed in cancer tissues other than prostate, RNAs from six each of both frozen normal and cancer tissues of breast, colon, lung, and bladder origins were obtained from the CHTN, and RNA was prepared as described above. Relative quantitative RT-PCR was performed to evaluate expression of the smaller UROC28 transcript, which was shown to be expressed in several tissues by Northern hybridization (Fig. 1). As shown in Fig. 7, the expression of *UROC28* gene was up-regulated more than 4-fold in breast cancer, and 2.5-fold in bladder cancer, when compared with corresponding normal tissues. However, the gene did not show significant differential expression in lung and colon cancers.

Regulation of UROC28 Expression by DHT. Because UROC28 is up-regulated in both prostate and breast cancers, and hormones similarly regulate both prostate and mammary glands, we tested whether the expression of this gene is regulated by androgen. LnCaP cells were initially culture in RPMI 1640 supplied with 10% charcoal-stripped serum for 48 h; then different amounts of DHT (0, 0.1, 1, 10, and 100 nM) were added to the medium and incubated for 24 h. RNAs were then isolated and subjected to RT-PCR analyses. As shown in Fig. 8, expression of UROC28 mRNA is stimulated about 2- to 4- fold by DHT, and the stimulation is DHT dose dependent.

Chromosomal Localization of *UROC28* Gene. FISH chromosomal mapping was performed to determine the chromosomal localization of *UROC28* gene. As shown in Fig. 9, based on the match of DAPI banding and UROC28 FISH hybridization signal, UROC28 was mapped to the long arm of chromosome 6. The detailed position was further determined based on the summary from 10 photos (Fig. 9*C*), which mapped the gene to chromosome 6, region *q23-q24*. Both FISH mapping (Fig. 9) and human genomic DNA Southern hybridization (data not shown) indicated that a single copy gene encodes UROC28.

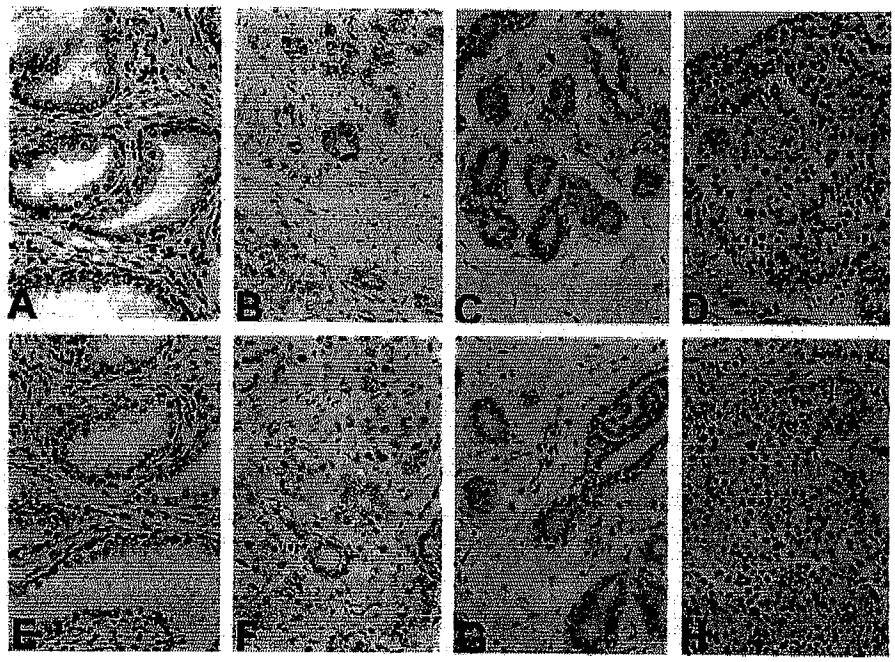

Fig. 5. Elevated levels of UROC28 protein and mRNA were detected in glandular epithelial cells of prostate and breast cancers. Immunostaining using UROC28-specific polyclonal antibody localized the UROC28 protein (*red immunostaining signal*) in the cytoplasm of glandular epithelia of (*A*) nonmalignant prostate acini, (*B*) prostate cancer of Gleason scores of 8, (*C*) nonmalignant breast tissue, and (*D*) metastatic intraductal breast cancer. Nuclear localization of UROC28 protein was also observed in prostate cancer epithelium (*B*). *In situ* hybridization on serial tissue sections using biotinylated UROC28-specific oligonucleotide probe colocalized the brown hybridization signal in glandular epithelia in (*E*) nonmalignant prostate acini, (*F*) Gleason score 8 prostate, (*G*) nonmalignant breast tissue, and (*H*) metastatic intraductal breast cancer. ×400.

DISCUSSION

We report the cloning of a novel gene that is overexpressed in prostate cancer and two other cancers (breast and bladder). The gene transcribes two mRNA variants that share most of their sequence including the whole open reading frame. They differ only at the end of the 3′ untranslated region. Whereas the small mRNA is expressed in most of the tissue types tested, the larger mRNA variant is confined mainly to prostate, with some expression in spleen (Fig. 1). In prostate, the expression level of the smaller transcript is about twice that of the larger transcript (Fig. 1). The fact that expression of the larger mRNA transcript is much higher in prostate when compared with other tissues implies a possible delicate control mechanism of the gene in this tissue. Because both transcripts are similarly up-regulated in prostate cancer (Fig. 4), they may be equally important in prostate carcinogenesis. The exact roles of the different 3′ untranslated regions of the two transcripts remains to be determined.

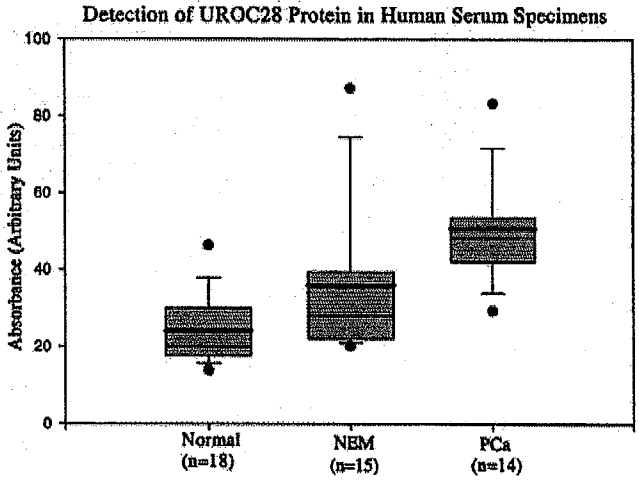

Fig. 6. Detection of UROC28 protein in serum samples by Western slot blot. *Solid bold horizontal lines in the box plots*, the mean serum UROC28 protein levels for each test group. *Also in the box plots*, the 5th percentile, median, and 95th percentile of the UROC28 protein levels. *Solid circles*, the minimum and maximum for UROC28 protein levels for each test group. Sera from prostate cancer patients with a clinical stage ranging from T_{1a} to T_4 and Gleason scores of 3–7 (average, 6) were included.

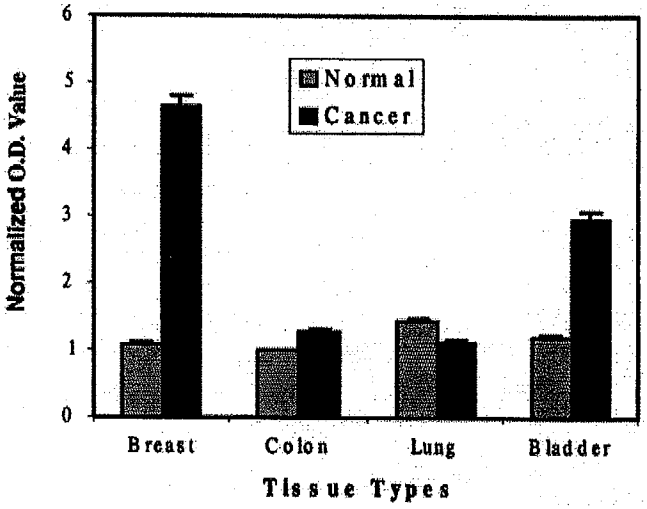

Fig. 7. Expression of UROC28 in various cancers. RNAs were isolated from six each of both frozen normal and cancer tissues of breast, colon, lung, and bladder origins and subjected to relative quantitative RT-PCR analyses to evaluate expression of the smaller UROC28 transcript. *β-actin* RT-PCR was performed on the same samples for normalization. All of the normalized values are presented as the mean ± SD.

The single copy of *UROC28* gene is mapped to chromosome *6q23–24* region. Genes in this chromosomal region that are associated with prostate cancer have been reported previously (27–29). Hyytinen *et al.* (27) showed that loss of *6q24-qter* was associated with androgen independence and tumorigenicity. Srikantan *et al.* found that loss of *6q23–24* might be associated with some prostate cancers (28). Cooney *et al.* indicated that the proximal *6q* deletions are related to prostate cancer progression (29). Furthermore, the *6q23–24* region has also been implicated in other cancers. For example, amplification of *c-myb* in *6q24* was shown to correlate with pancreatic tumor progression (30); loss of heterozygosity in this region was associated with progression of breast and cervical cancers (31–33). Using RT-PCR, *in*

situ hybridization, and immunohistochemistry, we found that the expression of both *UROC28* mRNA and protein is also overexpressed in breast and bladder cancers. The fact that UROC28 is overexpressed in multiple cancer types and that the gene is localized to chromosome *6q23–24* implies that the gene may represent another oncogene candidate in this large (megabases) chromosomal region. However, loss of heterozygosity studies are often used to locate tumor suppressor genes; thus, finding *UROC28*, an oncogene candidate, in a region of chromosomal loss is somewhat surprising.

The similar up-regulation of the gene in both prostate and breast cancers deserves special attention, because of some common characteristics of breast and prostate cancers. It is well known that the growth and proliferation of both breast and prostate cancer cells are modulated by androgens via common *androgen receptor* (*AR*; Refs. 34–36). Recently, several genes have been reported to be regulated similarly in these two cancers, including *AR* (34), *BRCA1* (37), *E-cadherin* (38), *PSA* (39), *FGF-1* (40), *EGFR* (41, 42), *HER2/neu* (10, 41, 42), and *Kai 1* (43, 44). Furthermore, expression of some of these genes is regulated by the same mechanism, such as hypermethylation (38), in both prostate and breast cancers. The up-regulation of *UROC28* mRNA and protein levels in both prostate and breast cancers and the stimulation of the gene by DHT further support the notion that similar pathways may be involved in modulating the growth and progression of these two cancers.

The correct diagnosis and prognosis of prostate cancer is critical in definitive and curative management of this disease. It is agreed that patients diagnosed early with organ-confined tumors are curable ~90–95% of the time with radical prostatectomy (45) or about 85–95% with radiation therapy (46). Current clinical diagnostic dilemmas created for prostate cancer detection surround the changing natural history of the disease produced by PSA screening (47, 48). There is a significant amount (~60–70%) of clinical stage T_{1c} disease (PSA >2.5 ng/ml and nonpalpable disease) presenting at diagnosis that has variable pathology present in the prostate organ (47, 48). The latter provides a new diagnostic and prognostic pretreatment challenge at the time of diagnosis in terms of providing a more precise determination of the extent of the patient's disease (45). The present widely used PSA assay cannot reliably distinguish between prostate cancer and BPH, nor predict which prostate cancer will progress rapidly. We have shown in this communication that the serum UROC28 protein level is significantly different between normal and BPH, and between BPH and prostate cancer individuals. These preliminary results suggest that UROC28 may provide an alternative serum marker either alone or in combination with other markers such as PSA for a more accurate diagnosis of prostate cancer. The wide range of UROC28 protein detected in the NEM sera might be attributable to the possibility that some of these NEM cases might have contained occult cancer, because an average of 25% of prostate cancer may be missed at the first biopsy (49). More studies are under way to further explore the potential clinical utility of UROC28 as a new serum marker for prostate cancer.

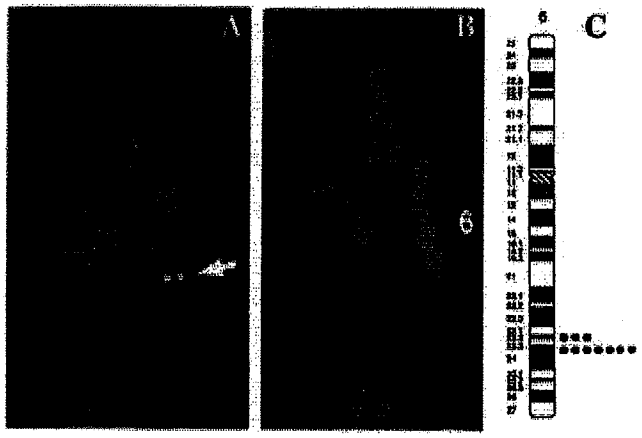

Fig. 9. UROC28 FISH chromosomal mapping results. *A*, the FISH signals on the chromosome; *B*, the same mitotic figure stained with DAPI to identify chromosome 6; *C*, diagram of FISH mapping results; •, the double FISH signals detected on human chromosome 6.

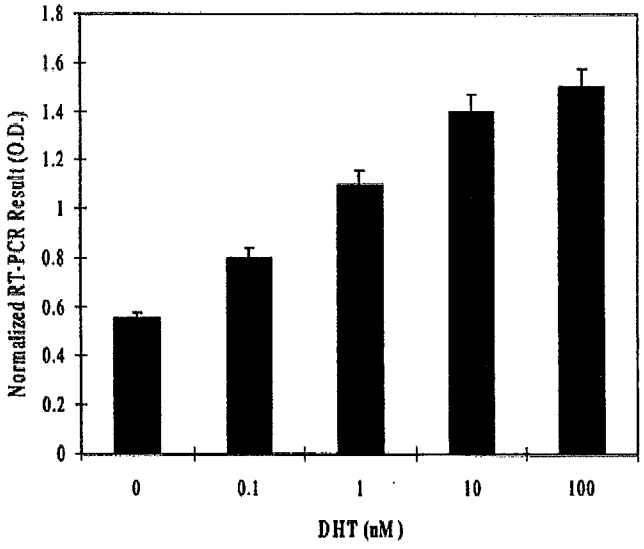

Fig. 8. Stimulation of UC28 expression by DHT in LnCaP cells. The cells were cultured in RPMI 1640 with 10% charcoal- stripped serum for 48 h first, then incubated in the same medium with indicated amount of DHT for 24 h. RNA was isolated and subjected to RT-PCR analyses. β-actin RT-PCR was performed on the same samples for normalization. All of the normalized values are presented as the mean ± SD.

In conclusion, we have demonstrated that the expression of *UROC28* gene is significantly up-regulated in primary as well as metastatic prostate cancer tissues, with higher expression of the gene observed in cancer tissues of higher Gleason scores and metastatic tissues. We also demonstrated that UROC28 protein could be detected in serum, and a higher serum UROC28 protein level was detected in prostate cancer individuals compared with normal individuals. Results from *in situ* hybridization and antibody immunostaining confirm that the gene is up-regulated at the mRNA and protein levels in the glandular epithelial cells of prostate cancer. Basal cells of the prostate acini have been referred to as progenitor cells for prostate glandular tissues. The loss of the basal cell layer and the overexpression of *UROC28* mRNA in cancer glandular epithelium may imply a regulatory role for UROC28 and basal cells in prostate carcinogenesis. The observation of nuclear localization of UROC28 protein in prostate cancer glandular epithelia may imply unique tissue-specific regulatory mechanism of UROC28. Our findings support the possibility that *UROC28* gene may play a role in prostate cancer progression, and that the increased expression of *UROC28* mRNA and protein may serve as potential new markers for better management of prostate cancer.

ACKNOWLEDGMENTS

We would like to thank Sheryl Christofferson and Lei Gong for providing technical support.

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EXHIBIT B

2. I am one of the inventors named on the present patent application. I am also a co-author on An *et al.*, *Cancer Research* 60:7014-7020 (2000), which is cited by the Examiner in the Office Action dated May 12, 2004.

3. I have an understanding of the specification of the application, the Office Action dated May 12, 2004, and An *et al.*, *Cancer Research* 60:7014-7020 (2000).

4. I understand that the Examiner has rejected current claims 1, 3, 4, 6, 9-10, 12-14, 21-23, 25, 35, 37-38, 65, 67-69, and 71-72 of the application on the grounds that they are not enabled by the disclosure in the specification. In making this rejection, the Examiner asserts that the data provided in the specification does not establish that UC28 is overexpressed on the membranes of primary prostate cancer cells. I am providing this information to show that the present specification does establish that UC28 is overexpressed on the membranes of primary prostate cancer cells.

5. The present specification discloses that UC28 expression is upregulated in cancer tissues over normal and benign tissues (Specification, p. 79, ln. 21-22). The present specification also discloses that a significant portion of UC28 protein localizes to the cell membrane (Specification, p. 114, ln. 12-13). Localization of the UC28 protein on the cell membrane of prostate cancer cells was demonstrated in the C4-2B cell line using a rabbit polyclonal antibody produced against a UC28 synthetic peptide and visualized by fluorescent confocal imaging technology (Specification, p. 114, ln. 4-13).

6. The present specification discloses the amino acid sequence of the UC28 protein in SEQ ID NO: 2. Amino acids 34 to 50 of SEQ ID NO: 2 encode a putative transmembrane domain.

The presence of this putative transmembrane domain indicates that UC28 localizes to the cell membrane.

7. The Examiner cites An *et al.*, *Cancer Research* 60:7014-7020 (2000) as evidence that the UC28 protein is primarily located in the cytoplasm, and thus not on the membrane, of primary prostate cancer cells. As mentioned above, I am a co-author on the An *et al.* paper. While An *et al.* states that "UROC28 protein was localized primarily in the cytoplasm of prostate and breast cancer glandular epithelial cells" (An *et al.*, p. 7017, col. 2), this does not contradict the disclosure in the present specification that a significant portion of UC28 protein localizes to the cell membrane.

8. An *et al.* characterized the distribution of UC28 in terms of nuclear localization versus cytoplasmic localization. This is evident when An *et al.* is read in context: "UROC28 protein was localized primarily in the cytoplasm of prostate and breast cancer glandular epithelial cells (Fig. 5A-D). However, distinct nuclear localization of UROC28 protein was also noted in prostate cancer glandular epithelia (Fig. B)." An *et al.*, p. 7017, col. 2. The use of conventional fluorescence microscopy limited the ability of An *et al.* to more specifically characterize the localization of UC28. In the present specification, however, confocal microscopy was used to visualize UC28 on the cell membrane of prostate cancer cells. Confocal microscopy permits the user to obtain sharply defined optical sections that eliminate or reduce fluorescence away from the focal plane.

9. In conclusion, the present specification discloses that the expression of UC28 is upregulated in cancer tissues over normal and benign tissues, and that a significant portion of UC28 protein localizes to the cell membrane. The amino acid sequence of the UC28 protein

disclosed in SEQ ID NO: 2 contains a transmembrane domain, which indicates that the protein localizes to the cell membrane. Furthermore, the localization of the UC28 protein on the cell membrane was confirmed in the prostate cancer cell line C4-2B. Thus, the present specification discloses that UC28 is overexpressed on the cell membrane of prostate cancer cells as compared to normal prostate cells. Finally, the evidence presented in the specification is not refuted by An *et al.*

10. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 11/12/04

Robert W. Veltri

Robert W. Veltri, Ph.D.

Exhibit 1 to Declaration of
Robert W. Veltri, Ph.D.

[October, 2004]

DEMOGRAPHIC INFORMATION

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Education and training (in chronological order):

B.A.	1961-63	Youngstown State University	Biology/Chemistry
M.S.	1963-65	West Virginia University	Microbiology
Ph.D.	1965-68	West Virginia University	Microbiology

Professional Experience (in chronological order):

Positions	Institutions	Dates
Assistant Professor & Director of Research	West Virginia University Medical School, Div. of Otolaryngology and Department of microbiology	1969-72
Associate Professor & Director of Research	West Virginia University Medical School, Div. of Otolaryngology and Department of microbiology	1972-75
Professor & Director of Research	West Virginia University Medical School, Div. of Otolaryngology and Department of microbiology	1975-81
Director of virology and special immunology fee for service WVU hospital diagnostic laboratory	West Virginia University Medical School and Department of Clinical Pathology	1972-81
Director of R&D;	Cooper- Biomedical, Inc., Malvern, PA	1981-84
Project Director	National Foundation for Cancer Research, Bethesda, MD	1981-86
President/CEO	American Biotechnology Company, Rockville, MD	1984-88
Executive Vice President & Chief Scientific Officer	Theracel, Inc., Rockville, MD	1988-90
Vice President of R&D & Chief Scientific Officer	CytoDiagnostics, Inc., OKC, OK.	1990-94

Vice President of R&D and General Manager of R&D	UroCor, Inc., OKC, OK.	1994-01
Adjunct appointee in the department of pathology	Oklahoma University Health Sciences Center, OKC, OK.	1994-01
Institutional Biosafety Committee	Oklahoma University Health Sciences Center, OKC, OK.	1997-01
Governor of State of Oklahoma Health Research Committee member	State of Oklahoma Center for Advancement of Science and Technology (OCAST)	1997-01
Biotechnology Advisory Committee	Oklahoma City Community College	1998-01
Biomedical Engineering Advisory Board of the Department of Engineering	University of Central Oklahoma	2000-01
Technical supervisor of UroCor Clinical Chemistry laboratory	UroCor, Inc.	2000-01
Visiting Associate Professor	Johns Hopkins University School of Medicine	2001-03
Associate Professor	Johns Hopkins University School of Medicine	2003-

RESEARCH ACTIVITIES:

1. Veltri RW and BE Kirk. An antiviral substance in the tissues of mice acutely infected with lymphocytic choriomeningitis virus. J Gen Virol., 10(1):17-27, 1971.

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INVENTIONS, PATENTS, COPYRIGHTS (pending awarded):

1. Szent-Gyorgyi A, Fodor G, Veltri RW. 2-Furylbutyrolactone derivatives, their preparation, compositions containing them, and their use for modulation of the immune system in mammals. ***Patent No. 4,518,611; Issued: May 21,1985***. Assigned to Theracel Corp., Rockville , MD.
2. Veltri RW, Maxim PE, Fodor G. 2-Furylbutyrolactone modulation of the immune system in mammals. ***Patent No. 4,518,611 issued 05-21-85***. Assigned to Theracel Corp., Rockville, MD.
3. Maxim PE and Veltri RW. Assaying for circulating immune complexes with labeled protein A. ***Patent No. 4,617,262, Issued October 14, 1986.*** Assigned to CooperBiomedical Inc., Palo Alto, CA.
4. Veltri, RW and Maxim PE. Method of treating inflammation in mammals utilizing ketobutyrolactones and furylbutyrolactones. ***Patent No. 4,883,813, Issued November 28, 1989***. Assigned to Theracel Corp., Rockville, MD.
5. Fodor G, Sussangkarn K. and Veltri RW. Condensation products of cyclic diketones and ascorbic acid as immunomodulatory agents. ***Patent No. 4,883,808 issued 11-28-89***. Assigned to Theracel Corp., Rockville, MD.
6. Fox, SW and Veltri RW. Microencapsulated antitumor agent. ***Patent No. 4,963,364, Issued October 16, 1990.*** Assigned to the inventors.
7. Veltri RW, Fodor G, and Sussangkarn K. Pharmaceutically useful furyl substituted dihydroxyethylbutyrolactones. ***Patent No. 5,102,909 issued 04-07-92***. Assigned to Theracel Corp., Rockville, MD.
8. Veltri RW and Fodor G. Pharmaceutically useful Michael addition products of unsaturated aldehydes and ketones and ascorbic acid. ***Patent No. 5,098,933 issued 03-24-92***. Assigned to Theracel Corp., Rockville, MD.
9. An G, O'Hara SM, Ralph DA and Veltri RW. Biomarkers and targets for diagnosis, prognosis and management of prostate disease. ***Patent No. 5,882,864 issued 03-16-1999***. Assigned to UroCor, Inc., Oklahoma City, OK.
10. Gang An and Robert W. Veltri Biomarkers and targets for diagnosis, prognosis and management of prostate diseases. ***Patent No. 5,972,615. Issued October 26, 1999***. Assigned to UroCor, Inc., Oklahoma City, OK.
11. Gang An, Veltri RW et al Biomarkers and targets for diagnosis, prognosis and management of prostate, breast, and bladder cancer. ***Patent Number 6,218,529 issued on 4/1/2001***., Assigned to UroCor, Inc., Oklahoma City, OK.
12. Garry M. Marley and R.W. Veltri. Method for Selectively Inducing Biomarker Expression in Urologic Tumor Tissue for Diagnosis and Treatment Thereof. ***Patent No. 5,856,112 Issued 01-5-1999***. Assigned to UroCor, Inc., Oklahoma City, OK.
13. Veltri RW, Miller MC, Bacus MP, Ashenayi K. A sextant core biopsy predictive mechanism for non-organ-confined disease status". Issued November 23, 1999. ***Patent No. 5,989,811, Issued 09-29-2000***. Assigned to UroCor, Inc., Oklahoma City, OK.
14. Robert W. Veltri, Michael P. Bacus, M. Craig Miller. Prediction of Prostate Cancer Progression by Analysis of Selected Biomarkers. ***Patent No. 6,025 128, Issued 02-15-2000***. Assigned to UroCor, Inc., Oklahoma City, OK.
15. Gang An and RW Veltri. A novel, prostate-specific gene for diagnosis, prognosis and management of prostate cancer. ***Patent No. 6,156,515 Issued December 5, 2000***. Assigned to UroCor.
16. Ralph DA, An G, and Veltri RW. Diagnosis of disease state using mRNA profiles in peripheral leukocytes. ***Patent No. 6,190,857 issued February 20, 2001***. Assigned to UroCor, Inc., Oklahoma City, OK.
17. An, Gang and Veltri, Robert. Prostate-specific gene for diagnosis, prognosis and management of prostate cancer. ***Patent No.*** **6,369,195** ***Issued April 9, 2002.*** Assigned to UroCor, Inc., Oklahoma City, OK.

INVENTIONS, PATENTS, COPYRIGHTS (pending awarded):

18. Veltri, Robert W., Ashenayi, Kaveh, Hu, Ying and O'Dowd, Gerard J. Neural network for cell image analysis for identification of abnormal cells. ***Patent No. 6,463,438 issued October 8, 2002.*** Assigned to UroCor, Inc., Oklahoma City, OK.
19. Veltri RW. *Trademark*, "**UroScore™**". Serial No. 7468233 registered January 16, 1996 to UroCor Inc., Oklahoma City, OK. Registration No. 1948820 for Laboratory research services in the field of oncology.
20. Veltri RW. *Trademark*, Quantitative Nuclear Grade "**QNG™**". Serial No. 76173213 registered March 19, 2002 to UroCor Inc. of Oklahoma City, OK. Registration No. 2549632 for Medical services for detecting, staging, and prognosis of cancer.

Extramural Sponsorship (current, pending):

FUNDED SPONSORED RESEARCH:

"Evaluation of *UROC28* serum biomarker for early detection of prostate cancer." Proposed dates are April 1, 2003 through March 31, 2004. The proposal is in collaboration with an EDRN member, Dr. Alan W. Partin. $100,000 (Total for grant period – 1 year). I am the P.I. for the project and received Associate Membership in the EDRN. 35% effort. Started April 1, 2003.

"Epidemiology and significance of bladder cancer in areas endemic for *Shistosoma haematobium* in Africa". P.I. from JHU School of Public Health is Clive Schiff, Ph.D. Total Direct Costs for my role as co-investigator = $11,415.50. I have a 2.5% commitment to the grant. Started September 2003.

"Phase II trial of soy prior to radical prostatectomy". NIH is the sponsor and the program I.D. number is PAR-03-055 Quick-Trials for novel cancer therapies. P.I. from NYU is Maarten Bosland, DVSc, Ph.D. Total direct costs = $500,000; Current year direct costs = $250,000; Co-investigator at 5% effort and Direct costs for my project in year 1 are $21,453. Project to start April 1, 2004.

PENDING SPONSORED RESEARCH: None

PREVIOUS SPONSORED RESEARCH:

West Virginia University School of Medicine:

"Etiopathogenesis of recurrent serous otitis media." 1971-74; Deafness Research Foundation; Total award = $33,000; P.I. and 25%.

"Search for new human lung tumor associated antigens." 1974-78; National Cancer Institute Research Contract NO1-CB-43890. Total award = $422,909; P.I. at 25%.

"Lewis lung carcinoma immunotherapy model". 1976; American Cancer Society; total award = $2,544; P.I. at 5%.

"Epstein-Barr virus effects on the immune system." 1977; American Cancer Society; totals award =$2,550; P.I. at 5%.

"Clinical and viral studies of sudden hearing loss." 1978-81; NIH 1-R01-NS-15629-01; William Wilson M.D. (Massachusetts General Hospital) and Robert W. Veltri, Ph.D. (WVU), Co-principal investigators); Total award to WVU = $73,074.
"Immunomodulatory factors in head and neck cancer." 1981-83: National Cancer Institute 1-RO1-CA37390-01; Total award $148,000; P.I. 25% commitment. This NCI grant was transferred from WVU to industry (CooperBiomedical Inc.) in 1981.

American Biotechnology Co. (Theracel Corporation)

"Cloning and propagation of human tumor cells on interfaces of oil microcarrier emulsions." 1985-86; Topic 46; NIH-SBIR-Phase I; Co-P.I. with Dr. Ivar Giaver, Ph.D. at General Electric, Inc. $50,000.

"Effect of MFBLs on monokine and lymphokine production." 1985-86; Topic 29; NIH-SBIR Phase I; Baseler, M, Veltri RW and Maxim PE Co-Principal investigators; $50,000.

"Development of new synthetic immunoaugmentive agents." 1985-86; Topic 29; NIH-SBIR Phase I; Veltri RW, Baseler M and Maxim PE, Co-Principal Investigators; $50,000.

"Effect of MFBL on B-lymphocyte function." 1985-86; Topic 29; NIH-SBIR Phase I; Maxim PE, Veltri RW, and Baseler M, Co-Principal Investigators; $50,000.

"Antiviral efficacy of butyrolactone immunomodulators." 1988; Topic N87-4; Navy-SBIR Phase I; Veltri RE and PE Maxim Co-P.I.s; $50,000.

"Antiviral efficacy of butyrolactone immunomodulators." 1988-90; Topic N-87-4; N00014-89-C-0021; Veltri RE and PE Maxim Co-P.I.s; 25% commitment; $500,000.

CytoDiagnostics Inc. (UroCor Inc.)

"Nuclear matrix proteins and actin: biomarkers for cancer." 1992; Topic 12-L; Veltri RW and Briggman JL Co-P.I.s; $50,000.

"Neural networks to detect and classify bladder cancer" 1992; Veltri RW (P.I.) with RE Hurst of Oklahoma University Health Sciences Center and Kaveh Ashenayi of Tulsa University Department of Electrical Engineering (Co-investigators); $50,000.

"Combined use of tissue morphology, neural network analysis of chromatin texture and clinical variables to predict prostate cancer aggressiveness from biopsy material." 1998-00; Partin AW (P.I.) and Veltri RW (Co-investigator and subcontractor). Total direct costs for UroCor over 2.5 years was approximately $130,000.

"Diagnostic soluble urine test for interstitial cystitis." 1998-99; Veltri RW (P.I.); SBIR Phase I grant No. 1R43-DK53150-01A1; $100,000.

"Development of solution-phase Methylation Specific PCR to amplify and detect methylated target genes in isolated genomic DNA." 1999-00; An, Gang (P.I. and Veltri RW (Co-P.I.); SBIR Phase I grant No. 1 R43 CA90007-01; $100,000.

"Diagnosis and prognosis of prostate cancer using multiplex RT-PCR." 1999-00; An, Gang (P.I. and Veltri RW (Co-P.I.); SBIR Phase I grant No. 1 R43 CA83607-01; $100,000.

"p53 mutational analysis for bladder cancer prognosis." June 2000-May 2002; Ralph DA and James Prescott (Co-P.I.s) and Veltri RW (Co-investigator); SBIR Phase II No. 2 R44 CA76823-02Total award $693,828. Dr. Veltri served as the grant fiscal and technical administrator also assisted in obtaining IRB approval and setting up the IRB approved multi-site (Six) clinical trials for the project. The project was completed by Dr. James Prescott (New P.I.) after Dr. Veltri left in November, 2001.

UNDERGRADUATE AND GRADUATE TEACHING:

1968-72 - Nursing microbiology Course #26

1972-81 – Medical Microbiology Course #301, Virology Section consisting of 14 lectures, 4 labs and 2 clinical programs.

1975-77 – Course coordinator for the Medical Microbiology #301 course for medical students.

1972-81 – Clinical Laboratory Virology 491, four hours credit for graduates students in microbiology.

1974-81 – Basic Microbiology II Course No. 317-A. A six credit course. Dr. Veltri taught 2 credits of the six credit course dealing with the mechanisms of pathogenesis of viral diseases.

1970-81 - WVU continuing medical education lecturer for Neurology, Internal Medicine, Otolaryngology, Oncology, and Dentistry.

1972-81 – Established and sustained the WVU Sigma Xi Research Society multidisciplinary competitive graduate student research convocation and awards program.

1970-81 – A total of 19 Medical and Dental students rotated through my research laboratories on summer research fellowships for periods of time average 10-12 weeks.

1970-81 – Course Instructor at the annual meeting of the American Academy of Ophthalmology and Otolaryngology (AAOO) with Phillip M. Sprinkle, M.D. (Chairman of Otolaryngology) Course was entitled "Clinical immunology and Infectious Diseases in Otolaryngology".

GRADUATE MENTORING: *1970-83 - (primary advisor):*

James McClung, 1973-75; MS in microbiology; Thesis: "Epstein-Barr virus in human tonsil derived lymphocytes".

William Wainwright, 1973-75; MS in microbiology; Thesis: "Isolation and identification of early antigen complex from lymphoblastoid cell lines."

Louis Heyl, 1974-76; M.S. in microbiology; Thesis: "Epstein-Barr virus genome carrying lymphocyte subpopulations."

Maria Urquilla, 1974-76; M.S. in microbiology; Thesis: "In vitro production of soluble lung tumor associated antigens."

John McKolanis, 1975-77; M.S. in microbiology; Thesis: "Antigenic studies of the murine Lewis Lung tumor; An animal model for lung cancer."

Valerie A. Kikta, 1976-79; M.S. in microbiology; Thesis: Mechanism of action of serum blocking factor (SBF) isolated from infectious mononucleosis sera."

John L. Sloyer, 1970-72; Ph.D. in microbiology; Dissertation: "Immunobiology of human tonsil derived lymphocytes in vitro."

Lee Tuckwiller, 1972-79; Ph.D. in microbiology; Mechanisms of immunity in Herpes Simplex virus infections of man."

William Wainwright, 1975-80; Ph.D. in microbiology; Dissertation: "Immune regulatory mechanisms associated with Epstein-Barr infectious mononucleosis."

John R. McKolanis, 1977-80; Ph.D. in microbiology; Dissertation: "Immune response to a solublized membrane antigen of murine Lewis Lung Carcinoma."

Kenneth Dowler, 1980-82; Ph.D. in microbiology; Dissertation: "Fc receptors on the surface of Herpes Simplex viruses Types 1 and 2."

R. Scott Fritz, 1980-83; Ph.D. in microbiology; Dissertation: "Immunological investigations with monoclonal antibody specific for Lewis Lung tumor associated membrane antigen."

EDITORIAL ACTIVITIES (Reviewer)

British Journal of Cancer

Cancer Epidemiology, Biomarkers, and Prevention.

Cancer

Cancer Research

Clinical Cancer Research

Clinical Prostate Cancer

J Urology

Molecular Genetics and Metabolism

The Prostate

Urology

ORGANIZATIONAL ACTIVITIES:

Institutional Administrative Appointments:

1974-76 – WVU Foundation Fellowship Committee.

1974-81 – Clinical Cancer Education and Cancer Committee.

Professional Societies:

1973-75 – Treasurer WVU Chapter of Sigma Xi.

1976-77 – President WVU Chapter of Sigma Xi.

1978 – Member at large, West Virginia American Cancer Society Board of Directors.

1978-80 – Board of Directors, Monongalia County Chapter of the West Virginia Chapter of the American Cancer Society. Also, Public Speaking Chairman from 1979-80.

1985 – Secretary-Treasurer, Association of Biotechnology Companies, Washington, DC (Bruce Mackler, Ph.D. and J.D.; President and co-founder).

Membership: AACR, FASEB (AAI), ASM, American Academy of Microbiology, AUA, AAAS, AACC, Society for Basic Research in Urology (SBUR).

Conference Session Chair;

Invited by Dr. Ronald Berezney to chair a session for the FASEB summer conference meeting on "Nuclear Structure in Cancer" to be held in Saxton River, VT June 7-12, 2003.

Advisory Committees and Review groups:

2003 - Appointed to serve on the NIH/NCI SPORE Biomarker Development Working Group by Jorge Gomez, Chief, Organ Systems Branch, National Cancer Institute.

2003- Appointed to serve on the NIH/NCI INTERPROSTATE Biomarkers Study (IPBS) Group by Jorge Gomez, Chief, Organ Systems Branch, National Cancer Institute.

2003 Served on the NCI Genitourinary SPORE review committee – April 26-28, 2003.

2002-05 - Serving on the External Advisory Committee for the NIDDK BPH-MTOPS Prostate Samples Analysis (mPSA) consortium project (2002-2005).

1982 - Advisor to the National Committee for Clinical Laboratory Standards (NCCLS), Area Ligand Committee on Immunology and Ligand Assays.

Consultantships:

1971-74 – Immunology and microbiology consultant to Dr. Lee Brown of the **Dental Branch, Texas Medical Center**, University of Texas, Houston, TX. Project 1 was a NASA contract to monitor immune function and microbiological changes in astronauts in the Skylab project and Project 2 was a NCI research contract investigating dental caries etiology in Head and Neck cancer patients undergoing radiation therapy.

1985-86 – Consultant to Michael K. Ullman, Senior V.P. of **Cooper Development Inc.**, Therapeutics Division, Menlo Park, CA. The company was working on liposomes as a drug delivery system for cancer drugs.

Consultantships (Continued):

1987-88 – Consultant to **Mylan Pharmaceuticals** regarding the technical data for a submission of a potential FDA approved composition of matter (small molecule) for a new use as an anti-HIV agent to the FDA. I was responsible for analytical assessment of all pre-clinical antiviral data and preparation of a final report and recommendation.

2000 – Served as a consultant to **Qiagen** at a two day meeting. I served as a member of an Advisory Panel to review existing and new molecular-based technologies in development and also provided a lecture on the UroCor Inc. non-isotopic RNase Protection Assay (NIRCA) to assess mutations in p53 in urine samples of patients being monitored for bladder cancer recurrence.

2000 – August 1, 2000 I served as a consultant to JoAnn Boland and Dan McLaurin of **Becton-Dickenson,** diagnostics division, to review their Melastatin-DIG probe in-licensed from Millennium Pharmaceuticals. Their intent is to release an Analyte Specific Reagent (ASR) early in 2001. I reviewed the technical R&D data files and attended an Advisory Board meeting on the subject. A written technical report was also filed.

2000-01 – I served as a consultant to **Baylor College of Medicine Technologies** (BSMT) group. I evaluated the pre-clinical technology, which was to serve as a basis for a new start up in the area of prostate cancer therapeutics. The start-up Company is to be called Progression Therapeutics Inc. (PSi) and utilizes technology that originated in the BCM. I reviewed the entire technology and filed a go-no go report. Next, I assisted in preparing the back ground for the technical component of a business plan. This consultantship was approved by the JHU Conflict of Interest Office and the Department of Urology.

2001– 03 - Served as a speaker for the **Bayer Corporation,** Diagnostics division, in the area of PSA testing. I provided a total of six lectures during the year to urologists, pathologists and clinical chemistry laboratory specialists throughout the United States. This consultantship was approved by the JHU Conflict of Interest Office and the Department of Urology.

2003- Served as a speaker and consultant to the **GenProbe** scientific advisory board to review Molecular Oncology as a new diagnostic business opportunity.

RECOGNITION:

Awards and honors:

1976 – WVU School of Medicine outstanding teacher award

1979 – Elected a Fellow of the American Academy of Ophthalmology and Otolaryngology

1981 – Visiting professor of microbiology at the University of Chile in Santiago from July 28-August 8, 1981. I was one of three faculty members that taught a course in

viral oncogenesis and tumor immunology. My portion of the course consisted of eleven lectures, two seminars, and three laboratory sessions. Also, the faculty made me an honorary member of the Chilean Society of Microbiology.

1975-76 – President of the WVU Chapter of Sigma Xi, a research honorary. Initiated a interdisciplinary research competition in science for graduate students in the sciences.

2001 – Certificate of Recognition from Governor Keating for his services in helping to advance science and technology in Oklahoma presented by Dr. William A Sibley, President of Oklahoma Center for the Advancement of Science and Technology (OCAST) on October 30, 2001. Also a letter of appreciation was included from Dr. Kathy Kocan on behalf of the Health Research Committee.

Invited Talks and Panels:

1999 – Invited speaker at the Workshop on Chemoprevention of Prostate Cancer held August 8-9, 1999 in Baltimore, MD. Conference chairman was Ronald Lieberman, M.D. My talk was entitled "*Computer-assisted analysis of predictive factors and potential new biomarkers and methods for chemoprevention of prostate cancer.*" A manuscript resulted from this presentation: Veltri RW, Miller MC, and An G. Standardization, analytical validation, and quality control of intermediate endpoint biomarkers. *Urology* **57(Suppl 4A)**, 164-70, 2001.

2000 – Invited participant in the Superficial Bladder Cancer State of the Science (SOTS) Workshop held September 21-22, 2000 in Bethesda, MD. I participated in two breakout groups; Group A on Diagnostics/Markers/Endpoints and Group B Relapse and Progression, Therapeutics strategies and chemotherapy and immunotherapy.

2001 - Principal speaker at the summer FASEB summer conference on "Nuclear Structure and Cancer" co-chaired by Drs. Garry S. Stein, Ronald Berezney and Robert Getzenberg and held in Saxtons River, VT August 11-16, 2001. Lecture was entitled "Quantitative Nuclear Grade (QNG): A new image analysis-based biomarker of clinically relevant nuclear structure alterations." A manuscript resulted from this presentation: Veltri, RW, Partin, AW, and Miller, MC. Quantitative nuclear grade (QNG): A new image analysis-based biomarker of clinically relevant nuclear structure alterations. *J. Cellular Biochemistry*, **35:** (Suppl), 151-157, 2000.

2001 – Invited speaker at the 2001 MDACC "Tumor Markers: A new Era" meeting held from March 3-5, 2000 in Santa Barbara, CA. I conducted a roundtable session entitled " *Bladder Cancer Cytology Biomarkers for Detection and Monitoring: An Overview.*" Dr. Herbert Fritchie was the meeting organizer and coordinator.

2002 – Invited principal speaker at the 34th Oak Ridge Conference sponsored by the AACC and held April 25-26, 2002 in San Diego, CA. The title of my presentation was "A comparison of logistic regression statistical and artificial neural network modeling for prediction of prostate cancer pathologic stage." This presentation resulted in a manuscript published in: *Clin Chem.* 48(10):1828-34, 2002.

2003 - Principal speaker at the summer FASEB summer conference on "Nuclear Structure and Cancer" co-chaired by Drs. Ronald Berezney and Robert Getzenberg and held in Saxtons River, VT June 7-12, 2003. I chaired a Workshop entitled " Clinical Studies of Nuclear Structure and Cancer" and gave one of the lectures. The lecture was entitled "Nuclear morphometric image analysis: New applications in urological cancer."

Invited Reviews and Editorials:

1998 – A special issue of *Seminars in Urologic Oncology* dedicated to "Prognostic factors for Prostate Cancer", Alan W. Partin, M.D., Ph.D., Guest Editor. I was an invited

participant in the issue that was built around a single case report. My contribution was: Veltri, *RW*, GO O'Dowd, R. Orozco, M. Craig Miller, The Role of Biopsy Pathology, Quantitative Nuclear Morphometry, and Biomarkers in the Pre-operative Prediction of Prostate Cancer Staging and Prognosis. *Seminars in Urologic Oncology*, 16(3): 106-107, 1998.

Invited Reviews and Editorials:

1999 – Editorial - Invited to write a response to the article by A. de la Taille, Carl A. Olsson and Aaron E. Katz entitled "Molecular staging of prostate cancer: Dream or Reality"., Oncology 13: 187-94, 1998. My editorial was on 205 and 209.

2002 – An invited author for a special supplement of *Urology* dedicated to complexed PSA. I was invited to submit one article and served as a co-author on a second article. The articles were: 1) Veltri RW, Ph.D., M. Craig Miller. B.S. , Gerard J. O'Dowd. M.D., and Alan W. Partin, M.D., Ph.D. Impact of Age on Total and Complexed PSA Cutoffs in a Contemporary Referral Series of Men with Prostate Cancer. *Urology* 60 (Suppl 4A): 47-52, 2002 and 2) Ellison LE, Cheli C, Bright S, Veltri RW, and Partin AW. Cost-benefit analysis of total PSA, Free/Total PSA and complexed PSA for prostate cancer screening. *Urology* 60 (Suppl 4A): 42-46, 2002.

2003 – Prepared a invited review for Volume 2 ["Strategies for Cancer Chemoprevention:] of a two volume series entitled **"Cancer Chemoprevention"** being published by Humana Press and the Editors are Drs. Gary J. Kelloff, Ernest T. Hawk, and Caroline C. Sigman. My chapter is entitled *"Quantitative Nuclear Grade: The clinical applications of the quantitative measurement of nuclear structure using image analysis."*

OTHER PROFESSIONAL ACCOMPLISHMENTS:

From 1969-80 Dr. Veltri served on the faculty of microbiology and surgery of WVU, where his applied research included virology, immunology, and cancer biomarkers. Dr. Veltri developed a fee for service clinical immunology and virology diagnostic testing service for the WVU hospital's department of clinical pathology that generated about $100,000 in revenue by 1980. Teaching responsibilities included medical, dental, nursing and graduate level students in areas of microbiology, virology, and cancer biology. He also trained and served as advisor for six MS and six Ph.D. graduate students of microbiology during his tenure at WVU. A total of seventeen Medical, Dental, and Medical Technology students did research clerkships in Dr. Veltri's laboratory during his tenure at WVU. He obtained more than $1.0 Million in extramural research funding while at WVU.

In 1981-84 Dr. Veltri established an R&D team, which developed the first diagnostic application for liposomes that consisted of rapid (1-2 minutes) and sensitive (10-fold improvement to ng/ml) slide co-agglutination diagnostic kits for infectious and rheumatologic diseases. Three Class II, FDA approved 510K products were transferred to manufacturing and released to market based upon a proprietary and patented co-agglutination technology that employed a proprietary method to prepare protein-A coated Liposomes and combine them with antibody coated latex particles. The three FDA approved tests were for Streptococcal Group A, Rheumatoid arthritis, and Infectious Mononucleosis.

In 1984, Dr. Veltri founded the American Biotechnology Company in Rockville, MD (acquired by Theracel Corporation in 1988). We discovered two unique classes of L-Ascorbic acid derivatives based upon the use of an Aldol and Michael addition series of modifications performed at the C-2 position of the molecule (see patents 1,2,4,5, 7 and 8 above). These small molecules were all of very low toxicity, water-soluble and

demonstrated a variety of biological activities including anti-cancer, anti-infectious and anti-inflammatory activity.

OTHER PROFESSIONAL ACCOMPLISHMENTS:

In 1996, Dr. Veltri and his R&D team commercialized the first statistical-based (Logistic Regression model) and patented algorithm to predict prostate cancer post-operative stage based upon pre-treatment quantitative biopsy pathology as well as DNA ploidy and quantitative nuclear grade information determined by image analysis. The product was Trademarked "UroScore™", a term suggested by Dr. Veltri. There are three publications (reference # 51, 77 and 88 in the above bibliography) on the subject.

In 2002, Dr. Veltri accepted the appointment as Visiting Associate Professor of Urology at the Johns Hopkins University School of Medicine. He is currently directing a research Laboratory conducting research on diagnostic and prognostic biomarkers for urologic cancers using Quantitative Computer-Assisted Image Analysis to study nuclear structure and Proteomics technology (SELDI-TOF, 1D and 2D Electrophoresis, ELISA, Immunohistochemistry etc.).

In 2003, November 1, Dr. Veltri was appointed as a Full-time Associate Professor of Urology at The Johns Hopkins University School of Medicine. He continues to direct research on biomarkers for prostate, bladder and renal cancer using a image cytometry-based and proteomics-based approach.

EXHIBIT C

Results: Over a median follow-up of 45 months, 250 patients (50%) experienced disease progression after treatment, 49 (10%) developed distant metastases, 20 (4%) died from prostate cancer, and 21 (4%) died from other or unknown causes. The 4-year progression-free probability (PFP) was 45% (95% confidence interval [CI], 40%–50%). By multivariable analysis, predictors of progression were Gleason score of 8 to 10 (hazard ratio [HR], 2.6; 95% CI, 1.7–4.1; $P < .001$), preradiotherapy PSA level greater than 2.0 ng/mL (HR, 2.3; 95% CI, 1.7–3.2; $P < .001$), negative surgical margins (HR, 1.9; 95% CI, 1.4–2.5; $P < .001$), PSA doubling time (PSADT) of 10 months or less (HR, 1.7; 95% CI, 1.2–2.2; $P = .001$), and seminal vesicle invasion (HR, 1.4; 95% CI, 1.1–1.9; $P = .02$). Patients with no adverse features had a 4-year PFP of 77% (95% CI, 64%–91%). When treatment was given for early recurrence (PSA level ≤2.0 ng/mL), patients with Gleason scores of 4 to 7 and a rapid PSADT had a 4-year PFP of 64% (95% CI, 51%–76%) and of 22% (95% CI, 6%–38%) when the surgical margins were positive and negative, respectively. Patients with Gleason scores of 8 to 10, positive margins, and receiving early salvage radiotherapy had a 4-year PFP of 81% (95% CI, 57%–100%) when the PSADT was longer than 10 months and of 37% (95% CI, 16%–58%) when the PSADT was 10 months or less.

Conclusions: Gleason score, preradiotherapy PSA level, surgical margins, PSADT, and seminal vesicle invasion are prognostic variables for a durable response to salvage radiotherapy. Selected patients with high-grade disease and/or a rapid PSADT who were previously thought to be destined to develop progressive metastatic disease may achieve a durable response to salvage radiotherapy.

Commentary

The most effective and appropriate management of biochemical relapse following radical prostatectomy is a matter of much debate. Although previous investigators have suggested that isolated, local recurrence is uncommon after such surgery, this study suggests otherwise. Sixty-seven percent of patients achieved a complete response to radiation therapy and the 4-year progression-free probability was 45%. Furthermore, the study demonstrated that some patients whose cancers had features typically associated with distant disease, such as Gleason Grade 8 to 10 and a rapid PSA doubling time, had a durable response to radiation, especially if associated with a positive surgical margin. It should also be noted that the dose of radiation given in many cases may have been considered subtherapeutic by many so response rates could have been higher. In addition, this study was not designed to show that such therapy, even when effective, has an impact on disease-specific survival rates. Although not a randomized trial, physicians may want to rethink their patterns of care for such patients based on this study.

doi:10.1016/j.urolonc.2004.08.003

Peter R. Carroll, M.D.

Phase I trial of yttrium-90–labeled anti–prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ, Bander NH, *Division of Hematology and Medical Oncology, Department of Medicine, Weill Medical College of Cornell University, New York, NY.*

J Clin Oncol 2004;22:2522–31

Purpose: To determine the maximum-tolerated dose (MTD), toxicity, human antihuman antibody (HAHA) response, pharmacokinetics, organ dosimetry, targeting, and preliminary efficacy of yttrium-90–labeled anti-prostate-specific membrane antigen monoclonal antibody J591 (^{90}Y-J591) in patients with androgen-independent prostate cancer (PC).

Patients and Methods: Patients with androgen-independent PC and evidence of disease progression received indium-111-J591 for pharmacokinetic and biodistribution determinations followed 1 week later by ^{90}Y-J591 at five dose levels: 5, 10, 15, 17.5, and 20 mCi/m^2. Patients were eligible for up to three re-treatments if platelet and neutrophil recovery was satisfactory.

Results: Twenty-nine patients with androgen-independent PC received ^{90}Y-J591, four of whom were re-treated. Dose limiting toxicity (DLT) was seen at 20 mCi/m^2, with two patients experiencing thrombocytopenia with non-life-threatening bleeding episodes requiring platelet transfusions. The 17.5-mCi/m^2 dose level was determined to be the MTD. No re-treated patients experienced DLT. Nonhematologic toxicity was not dose limiting. Targeting of known sites of bone and soft tissue metastases was seen in the majority of patients. No HAHA response was seen. Antitumor activity was seen, with two patients experiencing 85% and 70% declines in prostate-specific antigen (PSA) levels lasting 8 and 8.6 months, respectively, before returning to baseline. Both patients had objective measurable disease responses. An additional six patients (21%) experienced PSA stabilization.

Conclusion: The recommended dose for ^{90}Y-J591 is 17.5 mCi/m^2. Acceptable toxicity, excellent targeting of known sites of PC metastases, and biologic activity in patients with androgen-independent PC warrant further investigation of ^{90}Y-J591 in the treatment of patients with PC.

Commentary

Prostate-specific membrane antigen (PSMA) is an excellent target for monoclonal antibody imaging and therapy because of its expression in high grade, metastatic and hormone-refractory prostate cancer [1,2]. In addition, it is not secreted like PSA or PAP. J591 is an anti-PSMA monoclonal antibody that binds tightly to the extracellular domain of PSMA. This is a significant improvement over previous monoclonal antibodies, which bound to an intracellular domain, available only in dead or dying cells. Toxicity in the current trial was consistent with that seen in other studies of ^{90}Y-labeled monoclonal antibodies and was mainly hematologic in nature. Two of the 29 patients with advanced

androgen-independent disease had significantly measurable responses. Six had stable serum PSA profiles. Importantly, 81% of bone and soft-tissue lesions were accurately targeted. This trial, showing acceptable toxicity, good targeting and evidence of biologic activity, suggests that additional investigation is warranted using different radionuclides, attachment of chemotherapeutic agents, and various trial designs.

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Laboratory Research

Platelet-derived growth factor receptor inhibitor imatinib mesylate and docetaxel: a modular phase I trial in androgen-independent prostate cancer. Mathew P, Thall PF, Jones D, Perez C, Bucana C, Troncoso P, Kim SJ, Fidler IJ, Logothetis C., *Department of Genitourinary Medical Oncology, Unit 427, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX.*

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Purpose: To study the platelet-derived growth factor receptor (PDGFR) inhibitor imatinib mesylate in androgen-independent prostate cancer (AIPC), alone and in combination with docetaxel, we designed a modular phase I trial. Our goals were to [1] evaluate the toxicity and maximum-tolerated dose of docetaxel with imatinib, and [2] evaluate the decline of prostate-specific antigen (PSA) induced by imatinib alone, and imatinib and docetaxel.

Patients and Methods: Twenty-eight men with AIPC and bone metastases were enrolled to receive imatinib 600 mg daily lead-in for 30 days, then imatinib 600 mg daily and one of six possible doses of docetaxel weekly for 4 weeks every 6 weeks.

Results: During the imatinib lead-in module, one dose-limiting toxicity (DLT) event was observed, while two (7%) of 28 had PSA decline (both <50%). With imatinib and docetaxel, cycle 1 DLT was found in three of 12 patients at docetaxel 30 mg/m^2, in three of four patients at docetaxel 45 mg/m^2, and in five of six patients at docetaxel 35 mg/m^2. DLTs (n = 40 total events) were principally fatigue (35%) and nausea (20%). Eight (38%) of 21 had PSA decline greater than 50%, and six (29%) of 21 had PSA decline less than 50%. Serial PSA declines beyond 18 months were observed. PDGFR-expressing tumor declined on serial bone marrow biopsies with combination therapy alone.

Conclusion: With imatinib 600 mg daily, the maximum-tolerated dose of docetaxel was determined to be 30 mg/m^2 weekly for 4 weeks every 6 weeks. Long-term responses were observed. The role of imatinib in modulating outcomes to docetaxel in AIPC is being tested in a randomized phase II trial.

Commentary

Imatinib (Imatinib Mesylate, STI571, or Gleevec™) is a small molecule tyrosine kinase inhibitor with established clinical activity in CML and GIST. Imatinib functions through competitive inhibition at the ATP-binding sites of tyrosine kinases. Imatinib has demonstrated preclinical activity in cell lines that express bcr-abl, the c-Kit receptor, and the platelet-derived growth factor (PDGF) receptor. PDGF receptor inhibition may result in activity against tumors such as breast cancer, prostate cancer, lung cancer, ovarian cancer, sarcoma, and gliomas. Combining imatinib with cytotoxic chemotherapeutic agents in the treatment of solid tumors might have particular advantages. Imatinib is known to modulate cytotoxic agent delivery to tumor cells, by decreasing the interstitial pressure and increasing the capillary to interstitium transport of selective drugs. This is mediated by inhibition of PDGF beta receptors expressed in blood vessels and stromal cells but not in malignant cells [1]. Increased capillary transport of chemotherapeutic agents can lead to enhanced antitumor activity. Because doceatxel has established activity in prostate cancer, combining imatinib with docetaxel may lead to enhanced antitumor activity based on the modulation of stromal cells and vascular endothelial cells by imatinib.

The above report combines weekly docetaxel with daily imatinib, and this regimen elicited a >50% PSA decline in 38% of the patients (n = 28). Notably, all the patients were heavily pretreated, with many of them having been previously treated with taxanes. Currently, this weekly regimen is being tested in the Phase II setting in chemo-naïve patients with AIPC. In another Phase I study [2], a total of 29 patients with advanced solid tumors were treated with daily imatinib and docetaxel every 3 weeks. The recommended Phase II (RP2) dose of this combination is: imatinib 400 mg po qd + docetaxel 60 mg/m^2 IV q 3 weeks. At this dose the combination is well tolerated, with pharmacokinetic analyses, revealing no drug-drug interaction. In this report almost half of the patients had AIPC, with equal numbers of chemo-naïve and chemo-refractory patients. In contrast to the first report, none of the taxane refractory patients (n = 6), had a PSA response to the q 3 week regimen. However, six of seven taxane-naïve patients had a >50% PSA response to this regimen, with a prolonged duration (3–9 months). Although the numbers of patients with AIPC were relatively small in both the studies, collectively these reports suggest that the combination of imatinib and doceatxel maybe useful in selected subsets of patients with AIPC.

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